

***Escherichia coli* populations from diabetic and non-diabetic patients with bacteraemia and faecal samples from healthy subjects – a comparative study**

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SUMMARY

Biochemical reactions, using the PhP-EC system of biochemical fingerprinting, were evaluated in order to group strains into different clusters and to investigate whether a biochemical typing system may be used to distinguish between randomly selected *Escherichia coli* strains obtained from bacteraemic patients and healthy controls. Fifty epidemiologically unrelated strains isolated from blood of non-diabetic patients and 46 faecal control strains were studied. Separately, 70 *E. coli* strains from 64 diabetic patients with bacteraemia were investigated. Diversity index was 0.977, 0.969 and 0.941 respectively. The strains were clustered at a similarity level of 0.95. The bacteraemic and control strains were subdivided into 14 different clusters with 2–12 strains each and 40 'outliers'. The largest cluster was dominated by bacteraemic strains (9/12, 75%). Two other clusters were dominated by control strains. In the remaining groups blood and faecal isolates were evenly distributed. No biochemical test was able to distinguish between bacteraemic and faecal control strains. Strains from patients with diabetes mellitus were grouped in 11 clusters containing 2–14 strains and 22 'outliers'. The low diversity index of bacteraemic strains obtained from diabetic patients as compared to other strains indicated a greater homogeneity. However, no correlation was observed between the examined host factors and the clusters.

INTRODUCTION

Gram-negative bacteraemia is a major infectious disease problem in modern medical centers [1]. It is largely an iatrogenic and nosocomial problem [2, 3]. *Escherichia coli* is one of the most common micro-organisms causing urinary-tract

infections [4, 5] and bacteraemia [6]. Patients with diabetes mellitus run an increased risk of acquiring Gram-negative bacteraemia, as compared to non-diabetics [7-9]. Epidemic outbreaks with *E. coli* have been described previously [10-13]. It has therefore been essential to establish simple and reliable methods to diagnose epidemic outbreaks and find markers indicating virulence.

E. coli is usually classified according to O, K and H serotype [14], with the aid of antibiogram [10, 13], biotyping [15, 16] and by its further evaluation biochemical fingerprinting [17]. These methods are well suited for testing large numbers of isolates. There are also other effective methods, although less adapted for screening, such as characterization of whole cell proteins [18], outer membrane proteins or plasmid content [19, 20].

Using the API 50 CH system, we have previously demonstrated that a few biochemical markers were associated with bacteraemia [21]. It cannot be ruled out, however, that the analysed strains were characteristic for the studied hospital and could partly be derived from nosocomial spreads of certain *E. coli* clones.

The aim of the present study was two-fold. Firstly, to investigate whether a biochemical typing system or specific biochemical markers may be used to distinguish between randomly selected bacteraemic *E. coli* strains and faecal isolates from healthy controls. Secondly, in diabetic patients with bacteraemia, to study biochemical characteristics of *E. coli* and investigate if certain host factors were associated with specific clusters of these bacteria.

PATIENTS AND METHODS

Diabetic patients with bacteraemia

Among hospital treated patients in the Stockholm area, a consecutive material was obtained of 70 *E. coli* strains, isolated from blood cultures of 64 diabetic patients. Forty-one were women and 23 were men, median ages 70 years, ranges 23-85 years and 23-82 years respectively. The majority had 1 bacteraemic episode during the study period, while 4 patients had 2 episodes and 1 patient had 3 bacteraemias. The patients were identified retrospectively and the cases were evaluated by review of their medical records. All patients were characterized according to type and duration of diabetes mellitus as well as anti-diabetic treatment. Signs of nephropathy (macro-proteinuria and/or increased serum creatinine) were considered as significant, if recorded at two or more occasions during at least 1 year prior to the onset of bacteremia. Episodes of proteinuria, in combination with signs of bacteriuria or urinary tract infection were not registered as indication of nephropathy.

Non-diabetic patients with bacteraemia

Altogether 50 epidemiologically unrelated *E. coli* strains were isolated in blood-cultures from bacteraemic patients, treated in 1983-7 in different wards at the Karolinska Hospital, Stockholm, Sweden. Thirty-one were women, mean age 62 years and 19 were men, mean age 67 years (ranges 19-85 years and 11-91 years respectively). Twenty-four patients suffered from malignant diseases, 8 had acute pyelonephritis and 5 hepato-biliary diseases. The remaining patients had no underlying diseases.

Controls

As controls served 46 faecal *E. coli* isolates obtained from healthy out-patients. Sixteen were women, mean age 51 years (range 17–80 years) and 30 were men, mean age 54 years (range 20–73 years). None of the controls had any gastrointestinal disorder. They had not experienced any infection or treatment with antibiotics within the last 3 months.

Microbiological investigation

Blood cultures were performed as previously described (22). Faecal samples were spread on CLED agar (Oxoid) and six colonies were selected, using a method that gives 99% probability that at least one colony belongs to the dominant aerobic faecal flora [23]. Gram-negative strains were identified by the API 20E system. All *E. coli* colonies in each faecal sample showed identical API patterns, and for further analysis only one colony was used.

PhP-EC system

The PhP-EC system (BioSys Inova, Stockholm, Sweden) consists of 24 biochemical reagents which have been selected to give high discrimination between independent *E. coli* strains [17, 24]. The dehydrated reagents are kept in flat-bottomed microtiter plates, with four sets of reagents for testing four isolates in each plate.

Calculations

The test results for all isolates were compared pairwise, and the similarity between each pair of isolates was expressed as the correlation coefficient. This resulted in a similarity matrix, for the randomly obtained bacteraemic and control strains, containing $96 \times (96 - 1)/2$ correlation coefficients. Likewise, the similarity matrix for the isolates from the diabetic patients contained $70 \times (70 - 1)/2$ correlation coefficients.

Discrimination capacity of the typing systems was calculated using Simpsons index of diversity (25).

The correlation matrix was clustered by the UPGMA method (26). Isolates with correlation coefficients above the identity level were assigned to the same biochemical phenotype. A similarity level of 0.95 was arbitrarily used to analyse whether bacteraemic isolates could be assigned to specific clusters.

RESULTS

Clustering and diversity index of strains from diabetic patients with bacteraemia

At a similarity level of 0.95 the isolates were subdivided into 11 different clusters containing between 2 and 14 strains each and 22 'outliers' (Fig. 1). The diversity index was 0.941. No correlation between any cluster and type of diabetes, proteinuria, serum creatinine, duration of diabetes mellitus, concomitant positive urine culture or mortality rate was observed. However, at an identity level of 0.97, three out of five patients with reinfections revealed biochemically

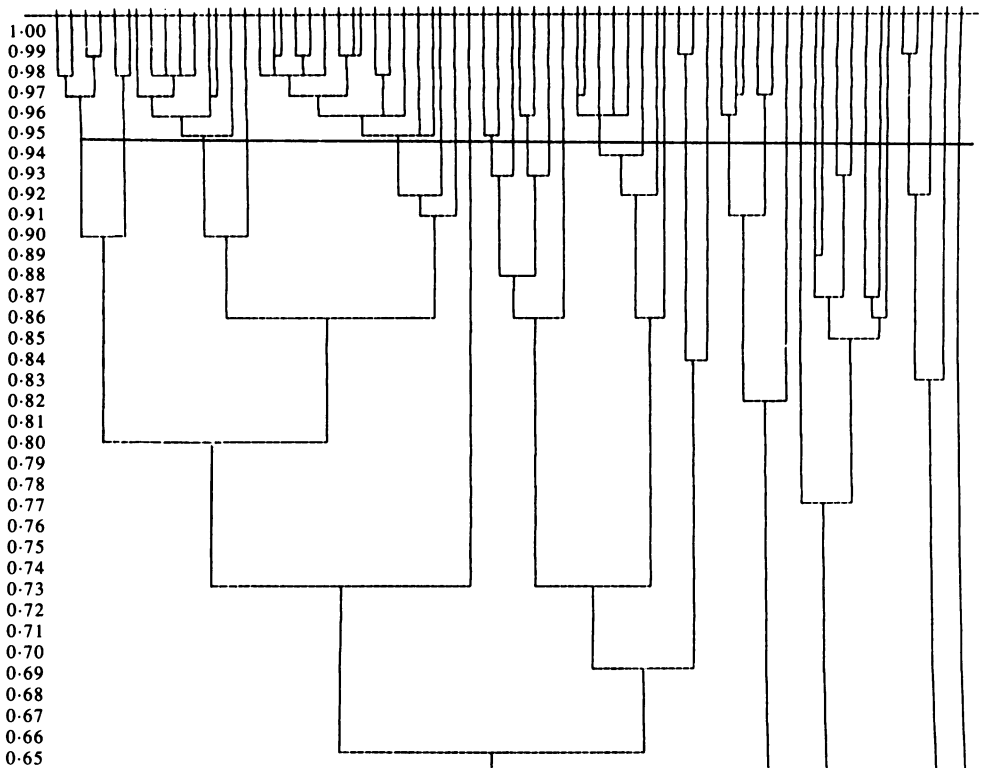


Fig. 1. Clustering of *E. coli* strains from diabetic patients with bacteraemia. Similarity level 0.95.

identical strains at the second bacteraemic episode. The time interval between the bacteraemic episodes was 18, 26 and 27 months respectively. The remaining two bacteraemias occurred after about 1 month.

Clustering and diversity index of strains from non-diabetic patients with bacteraemia and controls

At a similarity level of 0.95 the isolates were subdivided into 14 different clusters containing 2–12 strains each, and 40 ‘outliers’ (Fig. 2). The bacteraemic isolates were generally not associated with any particular cluster, although in the largest cluster (Table 1) 9 of 12 strains were bacteraemic isolates. Two other large groups contained 6 of 7 and 5 of 6 controls strains. In these two latter groups, the two bacteraemic isolates were obtained from patients with severe malignant diseases. In the remaining groups an even distribution among bacteraemic and faecal isolates was observed. The diversity index of the bacteraemic strains was 0.977 and of the control strains 0.969.

DISCUSSION

When clustering the test results from randomly obtained strains, the PhP-EC revealed certain clusters containing a majority of bacteraemic strains at a similarity level of 0.95. These strains could however not be associated with any

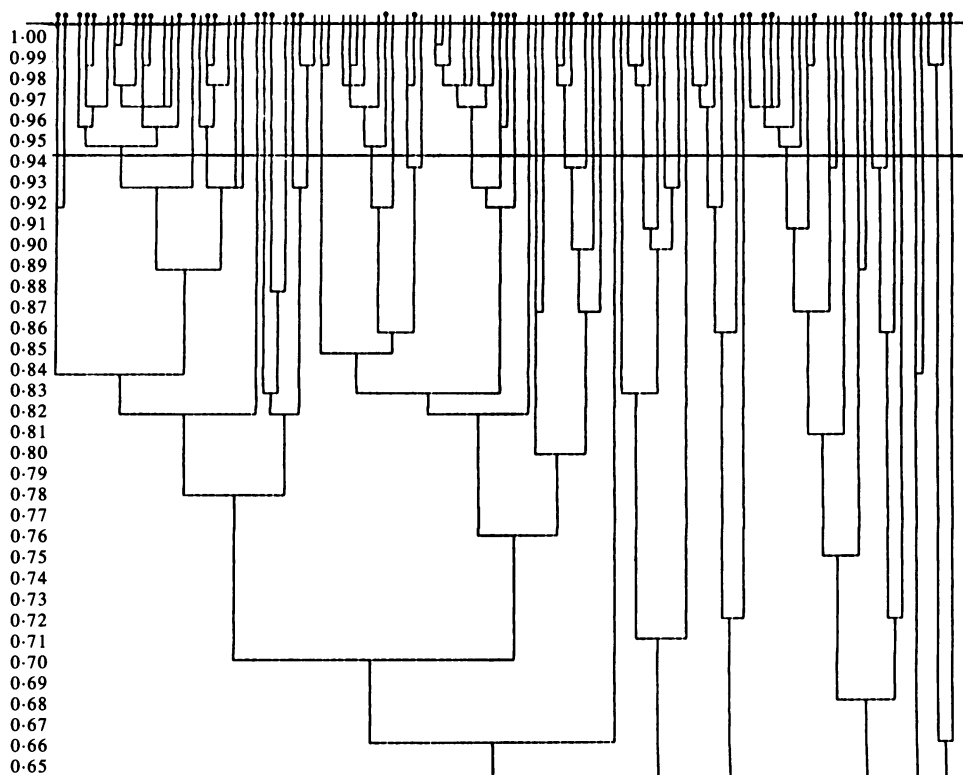


Fig. 2. Clustering of *E. coli* strains from epidemiologically unrelated non-diabetic patients with bacteraemia and from faecal control strains. Similarity level 0.95. ● indicates bacteraemic strains.

Table 1. Clustering of bacteraemic and faecal control strains according to *PhP-EC*-system. Groups containing more than three isolates

Group no.	No. of isolates	No. (%) of	
		Bacteraemic strains	Control strains
1	12	9 (75)	3 (25)
3	6	3 (50)	3 (50)
4	4	2 (50)	2 (50)
6	7	1 (14)	6 (86)
9	6	1 (17)	5 (83)

specific underlying disease. On the other hand, in clusters where faecal control strains dominated and single bacteraemic isolates were found, these strains were commonly isolated from patients with malignant diseases and impaired immune system. These results are in concert with our previous findings [9, 21, 27] that compromised patients more often become infected with normally low-virulent *E. coli* strains.

In diabetic patients none of the examined host factors was associated with specific clusters (data not shown). However, the diversity index among strains from diabetic patients was lower than among epidemiologically unrelated *E. coli*

isolates not only from controls but also from non-diabetic bacteraemic patients, indicating a greater homogeneity among the diabetic strains.

Three out of five diabetic patients with reinfections revealed a strain that was biochemically identical to the primarily identified strain. Two of these patients suffered from cholangitis while the third had a chronic pancreatitis. All three patients were appropriately treated with antimicrobial therapy at the first bacteraemic episode. Although the time interval between the infections was long, 18–27 months, it is likely that the initially infecting strain was not eradicated but could be harboured in the bile or possibly in the bowel.

Biotyping as a tool to diagnose virulent strains has been used by several investigators. Peeters and colleagues [28] reported on attaching and effacing enteropathogenic *E. coli* (AEEC) causing diarrhoea in suckling and weanling rabbits. In order to type the strains, they used the biotyping system originally described by Okerman and Devriese [16], involving fermentation of six different sugars. Similar results were obtained when two non-discriminating carbohydrates were omitted, leaving dulcitol, D-raffinose, L-rhamnose and sorbose. Determination of biotypes could thus be used to screen highly pathogenic AEEC. *E. coli* obtained from rabbits with diarrhoea were also studied by Camguilhem and Milon [29]. Serotyping was combined with fermentation of five different carbohydrates. In their study, serotype combined with fermentation of rhamnose was an important clue in the diagnosis of enteropathogenic *E. coli* strains. However, the above mentioned biochemical markers were probably associated with certain virulent *E. coli* clones, and not with virulence as such. It has been shown previously that bacterial clones may change their biochemical characteristics with time [30–32]. The use of only one or a few biochemical markers to identify virulent strains may therefore be hazardous.

In the present study we have deliberately chosen strains that were epidemiologically unrelated. This was supported by the high diversity index. The biochemical markers were evenly distributed among bacteraemic and control strains, and the bacteraemic strains were not assigned to any specific clusters. No particular test reagent could indicate virulence or distinguish between bacteraemic and control strains. These results indicate that the bacteraemic strains studied in the present investigation did not belong to any special virulent clones, but were merely *E. coli* from the normal human flora. Since almost half of the bacteraemic strains were obtained from patients with malignancies, it is also plausible that these patients were infected with normally low virulent strains. In a previous study [21] we observed that D-tagatose, saccharose, salicine and sorbose more often were fermented by bacteraemic strains as compared to faecal control strains. These data could not be confirmed by our present results and it cannot be ruled out that the strains from our previous study were partly derived from nosocomial spreads.

In conclusion, the PhP-EC system is a simple and reliable method to use for screening in epidemiological studies. In the present study, no biochemical marker was able to indicate virulence. Bacteraemic and faecal control strains could not be distinguished using the biochemical fingerprinting system. Among the diabetic patients no examined host factors were associated with specific clusters, nor were P-fimbriated *E. coli* strains assigned to certain clusters. The diversity index was

lower among strains from diabetics than randomly obtained bacteraemic and faecal control strains indicating a greater homogeneity among the former strains. Our results indicate that the bacteraemic isolates studied, randomly obtained and from patients with diabetes, did not belong to certain virulent clones and that bacteraemic strains *per se* do not harbour particular biochemical markers.

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REFERENCES

1. Young LS, Martin WJ, Meyer RD, Weinstein RJ, Anderson ET. Gram-negative rod bacteremia: microbiologic, immunologic, and therapeutic considerations. *Ann Intern Med* 1977; **86**: 456-71.
2. McCabe WR. Gram-negative bacteremia. *Adv Intern Med* 1974; **19**: 135-58.
3. Hale DJ, Robinson JA, Loeb HS, Gunnar RM. Pathophysiology of endotoxin shock in man. In: Proctor RA, ed. *Handbook of endotoxin*. Amsterdam: Elsevier, 1986: 1-17.
4. Winberg J, Andersen HJ, Bergström T, Jacobson B, Larsson H, Lincoln K. Epidemiology of symptomatic urinary tract infection in childhood. *Acta Paediatr Scand* 1974; **252** suppl: 1-20.
5. Jacobson SH, Källenius G, Lins LE, Svenson SB. P-fimbriated *Escherichia coli* in adults with acute pyelonephritis. *J Infect Dis* 1985; **152**: 426-7.
6. McGowan JE, Barnes MW, Finland M. Bacteremia at Boston city hospital: occurrence and mortality during 12 selected years (1935-1972), with special reference to hospital acquired cases. *J Infect Dis* 1975; **132**: 316-35.
7. Bryan CS, Reynolds KL, Metzger WT. Bacteremia in diabetic patients: comparison of incidence and mortality with nondiabetic patients. *Diabetes Care* 1985; **8**: 244-9.
8. Wheat LJ. Infection and diabetes mellitus. *Diabetes Care* 1980; **3**: 187-97.
9. Brauner A, Östenson C-G. Bacteremia with P-fimbriated *Escherichia coli* in diabetic patients: correlation between proteinuria and non-P-fimbriated strains. *Diabetes Research* 1987; **6**: 61-5.
10. Tullus K, Hörlin K, Svenson SB, Källenius G. Epidemic outbreaks of acute pyelonephritis caused by nosocomial spread of P-fimbriated *E. coli*. *J Infect Dis* 1984; **150**: 728-36.
11. Tullus K, Faexelius G, Fryklund B, Brauner A, Burman LG, Ransjö U. Outbreak of infections due to a P-fimbriated *Escherichia coli* O16:K1 in a neonatal intensive care unit. *Acta Paediatr Scand* 1988; **77**: 599-600.
12. Sweet AY, Wolinsky E. An outbreak of urinary tract and other infections due to *E. coli*. *Pediatrics* 1964; **33**: 865-71.
13. Wells CL, Podzorsky RP, Peterson PK, Ramsay NK, Simmons RL, Rhame FS. Incidence of trimethoprim-sulfamethoxazole-resistant Enterobacteriaceae among transplant recipients. *J Infect Dis* 1984; **150**: 699-706.
14. Ørskov F, Ørskov I. Serotyping of *Escherichia coli*. In Bergan T, ed, *Methods in microbiology*. Academic Press 1984: 44-112.
15. Gargan R, Brumfitt W, Hamilton-Miller JMT. A concise biotyping system for differentiating strains of *Escherichia coli*. *J Clin Pathol* 1982; **35**: 1366-9.
16. Okerman L, Devriese LA. Biotypes of enteropathogenic *Escherichia coli* strains from rabbits. *J Clin Microbiol* 1985; **22**: 955-8.
17. Kühn I. Biochemical fingerprinting of *Escherichia coli*. A simple method for epidemiological investigations. *J Microbiol Methods* 1985; **3**: 159-70.
18. Jackman P. Microbial identification and systematics based on whole-cell protein patterns. In: Sanna A, Morace G, eds, *New horizons in microbiology*. Elsevier Science Publisher B.V., 1984: 71-8.

19. Achtman M, Mercer A, Kusecek B, Pohl A, Heuzenroeder M, Aaronson W, Sutton A, Silver RP. Six widespread bacterial clones among *Escherichia coli* K1 isolates. *Infect Immun* 1983; **39**: 315-35.
20. Achtman M, Pluschke G. Clonal analysis of descent and virulence among selected *Escherichia coli*. *Ann Rev Microbiol* 1986; **40**: 185-210.
21. Brauner A, Boeufgras JM, Jacobson SH, Kaijser B, Källenius G, Svenson SB, Wretlind B. The use of biochemical markers, serotype and fimbriation in the detection of *Escherichia coli* clones. *J Gen Microbiol* 1987; **133**: 2825-34.
22. Brauner A, Leissner M, Wretlind B, Julander I, Svenson SB, Källenius G. Occurrence of P-fimbriated *E. coli* in patients with bacteremia. *Eur J Clin Microbiol* 1985; **4**: 566-9.
23. Carlsson B, Gothefors L, Ahlstedt S, Hanson LÅ, Winberg J. Studies of *Escherichia coli* O antigen specific antibodies in human milk, maternal serum and cordal blood. *Acta Paediatr Scand* 1976; **65**, 216-24.
24. Kühn I, Brauner A, Möllby R. Evaluation of numerical typing systems for *Escherichia coli* using the API 50 CH and the PhP-EC systems as models. *Epidemiol Infect* 1990; **105**: 521-31.
25. Hunter PR, Gaston MA. Numerical index of the discriminatory ability of typing systems: An application of Simpson's index of diversity. *J Clin Microbiol* 1988; **26**: 2465-6.
26. Sneath PHA, Sokal RR. Numerical taxonomy. San Francisco: Freeman and Company, 1973.
27. Jacobson SH, Tullus K, Wretlind B, Brauner A. Aerobactin mediated iron uptake by *Escherichia coli* strains causing acute pyelonephritis and bacteremia. *J Infect* 1988; **16**: 147-52.
28. Peeters J, Geeroms R, Ørskov F. Biotype, serotype and pathogenicity of attaching and effacing enteropathogenic *Escherichia coli* strains isolated from diarrheic commercial rabbits. *Infect Immun* 1988; **56**: 1442-8.
29. Camguilhem R, Milon A. Biotypes and O serogroups of *Escherichia coli* involved in intestinal infections of weaned rabbits: Clues to diagnosis of pathogenic strains. *J Clin Microbiol* 1989; **27**: 743-7.
30. Ørskov F, Ørskov I. Summary of workshop on the clone concept in epidemiology, taxonomy, and evaluation of the Enterobacteriaceae and other bacteria. *J Infect Dis* 1983; **148**: 346-57.
31. Kühn I, Franklin A, Söderlind O, Möllby R. Phenotypic variations among enterotoxinogenic *Escherichia coli* from Swedish piglets with diarrhoea. *Med Microbiol Immunol* 1985; **174**: 119-30.
32. Kühn I, Möllby R. Phenotypic variations among enterotoxinogenic O-groups of *Escherichia coli* from various human populations. *Med Microbiol Immunol* 1986; **175**: 15-26.