Untreated asymptomatic bacteriuria in girls: I—Stability of urinary isolates

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Abstract

Objective—To assess the frequency of spontaneous changes of bacterial strains in patients with untreated asymptomatic bacteriuria.

Design—Retrospective analysis of samples from all patients with renal scarring and random sample of patients with normal kidneys.

Setting—Outpatient clinic for children with urinary tract infections.

Patients—54 Girls aged 3·3-15·5 years with untreated asymptomatic bacteriuria caused by *Escherichia coli*.

Intervention—None.

End point—Change in bacterial strain.

Measurements and results—Serotyping and electrophoretic analysis of sequential bacterial isolates, representing 151 patient years of untreated asymptomatic bacteriuria. A total of 24 changes of strain were identified. Eleven were related to medical interference such as treatment of other infections with antibiotics.

Conclusions—Spontaneous changes of strain were uncommon, one change in 11·6 patient years, and thus are not a characteristic feature of the course of asymptomatic bacteriuria.

Introduction

In the pioneering studies of bacteriuria by Kunin and coworkers the recurrence rate after treatment was high, and 10% of the girls with one or more recurrences developed clinical pyelonephritis.[1] Subsequent investigations comparing treatment and non-treatment of schoolgirls with asymptomatic bacteriuria have not shown an increased risk for symptomatic episodes in the groups not given treatment.[2-5] Indeed, in a follow up study of non-treatment in 26 girls with asymptomatic bacteriuria and renal scarring there was no case in which the original bacterial strain caused acute pyelonephritis during an observation time of 72 patient years.[6] This suggested that a change of strain was required for the patient to develop pyelonephritis. Conversely, persistence of the same strain might be of advantage to the host by protecting against invasion by other bacterial strains.

The identification of strains of *Escherichia coli* isolated in sequence from one host as the same or different poses special technical problems. *E coli* are traditionally identified by surface antigens (O, K, or H serotype).[7,8] When asymptomatic bacteriuria was detected by screening, the isolates commonly could not be typed or were spontaneously agglutinating.[9] Furthermore, long term carriage of bacteria in the urinary tract seemed to induce changes in surface antigens (antigenic drift) with loss of serotype determinants.[9] Such a conversion from typable to non-typable may be difficult to differentiate from the acquisition of a new strain, and repeated findings of non-typable isolates in a patient do not necessarily imply persistence of the same strain. Alternative typing methods have recently been developed. Multilocus enzyme electrophoresis allows strains of *E coli* to be identified as accurately as by serotyping and has the advantage that all isolates can be identified.[10] We used multilocus enzyme electrophoresis in combination with determination of O antigens to assess the stability of strains of urinary bacteria in girls with untreated asymptomatic bacteriuria.

Patients and methods

The patients were part of a group followed at the outpatient clinic for children with urinary tract infections at the Children's Hospital, Gothenburg.[11] At the end of 1984 we had followed up 207 patients aged <16 years who had had untreated asymptomatic bacteriuria of at least three months' duration. From this group we included in the present study all patients with established renal scarring except one patient with an incomplete file (n=23) and 13 patients with normal kidneys who were randomly selected from the larger group. We also included 18 patients detected during a school screening programme who had had bacteriuria of at least three months' duration. Only patients with bacteriuria due to *E coli* were included. All 54 patients were girls and had a median age of 9·0 (range 3·3-15·5) years. The median duration of asymptomatic bacteriuria was 2·5 years (total 151 patient years).

Urine cultures were obtained from clean catch midstream urine specimens. Asymptomatic bacteriuria was defined by two cultures with growth of at least 100×10^6 *E coli*/l. The patients were examined shortly after diagnosis and subsequently every three to six months. The examinations comprised urine cultures, a careful history, and laboratory tests to detect affected kidneys according to a standardised protocol.[11] Bacteriuria cleared spontaneously in 14 girls during the follow up. Six received treatment after a period of untreated bacteriuria: two because of incontinence, two by mistake, one before an operation for reflux, and one because of fever that was probably of viral origin. Treatment with penicillin prescribed for other infections eradicated the bacteriuria in four girls and in one girl caused a change from *E coli* O9 to *E coli* O6, leading to the development of acute pyelonephritis. Twenty nine of the 54 patients still had bacteriuria at the end of the observation period.

All patients were investigated with intravenous urography. Renal scarring was defined as a local parenchymal reduction with deformity of the corresponding calix.[12] Voiding cystourethrography was performed in all but three patients. Vesicoureteric reflux was found in 14 of the 23 girls who had renal scarring, in two of whom the upper urinary tract was dilated. Four of the 31 girls with normal kidneys had reflux.

Bacterial isolates from urine were identified by

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standard techniques, and Gram negative bacteria were saved as deep agar stab cultures. Serotyping was done with antisera to known O antigens in an agglutination assay.[13] The isolates were designated as O typable or O non-typable. Spontaneously agglutinating isolates were grouped with other non-typable strains.

Multilocus enzyme electrophoresis is a procedure in which cytoplasmic proteins are separated according to their mobility in starch gel and detected by staining for enzymatic activity.[14] Slices of the gel were incubated with substrate for the enzymes coupled to histochemical stains. The 13 enzymes analysed were malate dehydrogenase, 6-phosphogluconate dehydrogenase, β-galactosidase, adenylate peptidase, phenylalanine-leucine peptidase, leucyl-leucyl-glycine peptidase, isocitrate dehydrogenase, phosphoglucose isomerase, aconitase, mannose-6-phosphate isomerase, glucose-6-phosphate dehydrogenase, alcohol dehydrogenase, and glutamic-oxaloacetic transaminase.

The position of each active enzyme appeared as a stained band in the starch gel, so that allelic variants of each enzyme (electromorphs) were detected by differences in mobility. Electromorphs for which enzymatic activity was lacking were defined as null alleles. Isolates were assigned to an electrophoretic type according to the combination of electromorphs for the enzymes tested.

The stability of the flora was analysed by using one isolate each year from each patient as well as all isolates before and after a registered change of O type. This included change from one O antigen to another, from O typable to non-typable, and from non-typable to O typable. In this way a total of 278 isolates from the 54 patients was analysed. Each isolate was compared with the next in the sequence, so that two consecutive isolates from one patient formed one pair. The prevalence of change of strains was evaluated by comparing the two isolates in all pairs (n=224).

TABLE I—*O type and multilocus enzyme electrophoretic type of isolates from yearly urine samples of one girl. Electrophoretic type I differed from type II in nine enzymes*

	1971	1972	1973	1974	1975
O type	7	7	NT	NT	NT
Electrophoretic type	I	I	I	II	II

NT=Non-typable.

TABLE II—*Appearance and disappearance of enzymatic activity in a sequence of isolates from one girl with untreated bacteriuria*

Isolate No	Enzyme* 1	2	3	4	5	6	7	8	9	10	11	12	13	O type
1	M	B	C	B	M+	A	B	•	S	•	M	B	B	18
2	M	B	C	B	M+	A	B	M	S	B	M	B	B	18
3	M	B	C	B	M+	A	B	M	S	B	M	B	B	NT
4	M	B	C	B	M+	A	B	M	S	•	•	B	B	NT
5	M	B	C	B	M+	A	B	M	S	B	M	B	B	NT
6	M	B	C	B	M+	A	B	M	S	•	•	B	•	NT
7	M	B	C	B	M+	A	B	•	S	•	M	B	B	NT

*1=Malate dehydrogenase, 2=6-phosphogluconate dehydrogenase, 3=β-galactosidase, 4=adenylate peptidase, 5=phenylalanine-leucine peptidase, 6=leucyl-leucyl-glycine peptidase, 7=isocitrate dehydrogenase, 8=phosphoglucose isomerase, 9=aconitase, 10=mannose-6-phosphate isomerase, 11=glucose-6-phosphate dehydrogenase, 12=alcohol dehydrogenase, 13=glutamic-oxaloacetic transaminase.
M, B, C, A, S, M+ are codes for different allelic variants of each enzyme
• = Unexpressed enzyme activity (null allele); NT=non-typable.

TABLE III—*Differences in characteristics of Escherichia coli isolates*

Electrophoretic type	O serotype: Same O type	Change of O type	O type changed to non-typable	Non-typable changed to O type	Both isolates non-typable	Total
No of pairs:						
Identical	39	0	24	7	56	126
With differences in null alleles	28	0	2	6	38	74
With differences in enzymes	0	4	4	6	10	24
Total	67	4	30	19	104	224

TABLE IV—*Number of bacterial strain changes per 100 patient years of asymptomatic bacteriuria related to renal scarring or vesicoureteric reflux*

	Observation time (years)	No of changes of strain: Spontaneous	Total
Patients with:			
Scarring	50	8	16
No scarring	101	9	16
Reflux*	56	5	14
No reflux	84	12	18

*Three patients were not investigated.

Results

A defined O type was found in 71 (31%) of the pairs. For the others multilocus enzyme electrophoresis was required to define a change of the strain. The power of multilocus enzyme electrophoresis to identify changes and the limitations of O typing are illustrated in table I. This patient carried an O7 strain from 1971 to 1972, but the isolates from 1973 to 1975 were not typable. Analysis by multilocus enzyme electrophoresis showed that one strain was carried from 1971 to 1973 and a new strain was acquired in 1974. The change was explained by the fact that the patient had been treated with penicillin between the samples taken in 1973 and 1974.

The use of multilocus enzyme electrophoresis was complicated by null alleles. Table II shows an example. The first and last isolates in the sequence from one patient expressed the same electrophoretic type. The isolates in between varied in the number of null alleles but no different enzymes were detected. As this pattern was seen in several of the patients, the appearance or disappearance of enzyme activity was not by itself regarded as a change of strain. Consequently, the definition of strain change in this study was based on a difference of at least one allelic variant in the enzymes expressed.

Table III shows that in 67 pairs of bacteria with the same O type no enzyme differences were observed. The four pairs which showed a change of O antigen showed a concomitant change in electrophoretic type. The remaining 153 pairs contained at least one isolate that could not be typed, and the possible change of strain could not be evaluated by O typing. Of the 30 pairs which changed from O types to non-typable, only four showed differences on multiple enzyme electrophoresis. Of the 19 that shifted from non-typable to O types, six differed by one or more enzymes; of 104 pairs consisting of two non-typable isolates, 10 showed a strain change on electrophoretic analysis. Thus the isolates differed in 24 (11%) pairs. The number of different enzymes ranged from one (four pairs) to nine (one pair).

During a total of 151 patient years we identified 24 changes of strain. In six girls the change coincided with antibiotic treatment given for other reasons, such as tonsillitis or otitis, and in four it occurred in connection with voiding cystourethrography. One girl was treated for bacteriuria by mistake and had an immediate recurrence without symptoms. The remaining 13 had no obvious explanation for the change of strain; it was therefore considered to be spontaneous. These spontaneous changes of strain thus occurred once every 11·6 patient years. The frequency of strain change was not increased in girls who had renal scarring or vesicoureteric reflux (table IV).

Among the 36 girls who had the same bacterial strain throughout the observation period (90 patient years), isolates with a defined O type were found at onset of

TABLE V—*Change of electrophoretic type of Escherichia coli related to changes of O type between first and last isolates from 54 girls with asymptomatic bacteriuria*

O serotype	No of patients with same electrophoretic type	No of patients with changed electrophoretic type
Same O type	15	0
Change of O type	0	1
O type changed to non-typable	10	5
Non-typable changed to O type	0	4
Both isolates non-typable	11	8
Total	36	18

bacteriuria in 25. In 15 of these girls the O type was continuously identified, whereas in 10 the O antigen was lost (table V).

Discussion

The present study was made possible by our follow up programme for children with urinary tract infections and our systematic storage of Gram negative bacteria.[11] The girls were seen regularly at the hospital as general practitioners in the city do not deal with children. They were seen at the hospital also for other diseases. Non-treatment of bacteriuria was first introduced in 1971 for patients without symptoms or renal scarring and was extended at the end of the 1970s to include those with known scars.[6]

Multilocus enzyme electrophoresis was originally used in studies of genetic relatedness in eukaryotes but has more recently been adapted to the study of bacteria.[14,15] The isoenzymes are products of chromosomal genes and have been mapped in *E coli* K-12.[16] An allozyme difference is caused by, for example, a change in the charge of an amino acid; it reflects a change in DNA. Though from different hosts, geographic locations, or sampling times, isolates that express the same electrophoretic type are likely to belong to the same clone. Multilocus enzyme electrophoresis and serotyping for O, K, or H antigens detect clones of *E coli* with similar accuracy provided that a complete serotype can be determined for these antigens. However, the limiting factor for O typing in a population with asymptomatic bacteriuria is the large proportion of strains that cannot be O typed. This was the case for 23 of the 54 isolates defined as the first in the sequences studied here. Of 24 strain changes identified by multilocus enzyme electrophoresis, only four were detectable by O typing. Ten were seen in pairs of bacteria converting from O typable to non-typable or vice versa, and in 10 pairs both isolates were not typable.

The longitudinal character of the present study allowed us to examine alterations of bacterial surface characteristics—for example, the O antigen—during long term bacteriuria. Ten out of 25 O typable strains converted to non-typable strains while retaining the same electrophoretic type. An alteration from O typable to non-typable had occasionally been seen in schoolgirls whose asymptomatic bacteriuria had been left untreated.[9] This suggested that the lipopolysaccharide structure was changed. Those early studies, however, lacked a bacterial indicator that could differentiate loss of surface antigen from change of strain. Our results show that loss of surface antigens (antigenic drift) occurs during long term exposure of *E coli* to the host environment in the urinary tract. As the completeness of the lipopolysaccharide influences virulence of the bacterium this adaptation usually results in less virulent strains.

Strain changes in patients with untreated bacteriuria have previously been proposed as a risk factor for further impairment of the kidneys.[17] We found no evidence for an increased frequency of change of strain among the girls who had renal scarring or vesicoureteric reflux. Nevertheless, change of strain carries a risk because of the potential for the development of symptomatic infection when a more aggressive strain is introduced: one of the patients in this study developed pyelonephritis after treatment with phenoxymethyl penicillin (see accompanying paper p 856). The low prevalence of spontaneous strain changes shown here supports non-treatment as a safe way of avoiding symptomatic urinary tract infections.

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