SHORT ARTICLE

Genomic identity of pyelonephritogenic *Escherichia coli* isolated from blood, urine and faeces of children with urosepsis

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Chromosomal genotypes of *Escherichia coli* isolates from blood, urine and faeces of infants with urosepsis were studied to find possible clonality of the isolates. The isolates were analysed by PCR for class I, II and III alleles of the pyelonephritis-associated adhesin gene *papG*. The macrorestriction profiles of the *papG*-positive isolates were analysed by pulsed-field gel electrophoresis and their O serogroups were determined. Genetically identical *E. coli* isolates from the blood, urine and faeces of the same infant were found in 8 of 10 infants. This finding confirmed the results of previous phenotypic studies that the reservoir of pyelonephritogenic *E. coli* is indeed the colon.

Introduction

The reservoir of pyelonephritogenic *Escherichia coli* is believed to be the colon. This hypothesis is based on old findings [1, 2], which showed that *E. coli* isolated from the urine of a patient suffering from a urinary tract infection (UTI) belonged to the same O serogroup as a strain isolated simultaneously from the faeces of the same patient. It was reported later that these urinary and faecal isolates expressed similar P-fimbrial adhesins [3], which are the major virulence factors of uropathogenic *E. coli*. The production of these adhesins is encoded by *papG* genes [4]. This study investigated whether *E. coli* isolates from blood, urine and faeces of the same child with urosepsis were genetically identical, as no such study analysing isolates from these three sources with new molecular methods has yet been published.

Patients and methods

The bacterial strains and background data originated in a prospective, open, multicentre study of 180 children with acute pyelonephritis (PN) [5]. Children (1–24 months old) with PN were studied. Informed consent

was obtained from the parent(s) or guardians at entry. The study protocol was approved by the institutional review board and the ethics committee of each participating unit. PN was defined by a positive urine culture (i.e., growth of $\geq 10^5$ cfu/ml in two consecutive sterile-bag specimens or any growth in a suprapubic bladder aspirate) and two out of three of the following criteria: C-reactive protein > 25 mg/L, fever $\geq 38.0^\circ\text{C}$, or pyuria (leucocyte count $> 10/\text{mm}^3$). Patients who had received antibiotic treatment within the preceding 2 weeks, and those with known congenital anomalies of the urinary tract or central nervous system-associated anomalies were excluded.

Blood cultures (148 of 180; 82%) were taken if indicated clinically. Both blood and urine samples were cultured by standard methods [6]. To collect faecal samples in a standardised manner, rectal swabs were taken. Chromogenic agar (Chromogenic *E. coli*/Coli-form Medium; Oxoid) plates that selectively allowed the growth and preliminary differentiation of *E. coli* were inoculated with the swabs. Blood, urinary and faecal *E. coli* isolates, as well as faecal primary cultures, were analysed by PCR for class I, II and III alleles of the pyelonephritis-associated adhesin gene *papG* [7]. To ensure the detection of *E. coli* carrying *papG* in faecal samples, growth from the first streaking area of the faecal culture and five separate *E. coli* colonies on the fourth streaking area of the culture plate were tested directly for *papG*. Of the *papG*-positive faecal cultures, the specific *papG*-positive

colonies were subsequently isolated and their identification was confirmed by standard methods [6]. The macrorestriction profiles of the *papG*-positive blood and urinary isolates and one faecal isolate were analysed further by pulsed-field gel electrophoresis (PFGE) after *Xba*I restriction of chromosomal DNA [8]. The isolates were O serogrouped by standard methods [9].

Results and discussion

Blood cultures yielded bacteria in 12 (8%) of 148 cases; *E. coli* was grown from 11 and *Enterobacter cloacae* from one. *Staphylococcus epidermidis* grew in two other blood cultures but they were considered to be skin contaminants and non-significant findings. Ten children with *E. coli* isolates present in the blood and urine also had faecal isolates available for further characterisation; one child had only one blood isolate available and, therefore, was excluded from the analysis. The serotype of all isolates from all three sources was identical for a given child; however, in two children all the isolates were O non-typable. The genomic fingerprinting by PCR for *papG* and PFGE revealed that all three isolates from all three sources from each individual child were genotypically identical in eight children (Table 1, Fig. 1). In faecal samples of eight children at least three of five separate colonies tested from the fourth streaking area of the culture plate were positive for *papG*. This finding suggests that *E. coli* with *papG* genes can be considered to represent major facultative flora of the intestine in these children. One child (no. 6) had a PCR *papG*-positive *E. coli* only from the growth taken from the first streaking area and in another child (no. 7) no *papG*-positive *E. coli* could be detected in faeces despite the highly sensitive PCR detection method used. This failure to detect *papG*-positive *E. coli* from the gut of one child may be because, in this child, *papG*-positive *E. coli* represented a minority population in the stools.

In the present study, the percentage of the positive blood cultures was low (8%) but similar to an earlier report (9%) [10] and higher than another (4%) [11]. In spite of the multicentre design of the study carried out in five paediatric hospitals, it was successful in

Fig. 1. PFGE banding patterns of (1) blood, (2) urinary and (3) faecal isolates from (a) child no. 9 and (b) child no. 10 with *E. coli* urosepsis. St, molecular size markers.

collecting urine and faecal samples from 10 blood culture-positive children on admission. Therefore, this material can be considered a representative sample of children with urosepsis.

Previous studies have shown that *E. coli* attaches by means of Gal α 1 $\rightarrow$ 4Gal β -containing receptors to colonic epithelial cells [12]. Also, it has been reported that children prone to UTIs carry P-fimbriate *E. coli* in their intestine more often than healthy controls [13]. P-fimbriate *E. coli* strains found in urine and faeces have been shown to be associated with bacteraemia in adults [14]. On the other hand, the clonal identity of the urinary and faecal strains has been reported in women with acute PN [15].

The present study showed for the first time that genetically identical isolates of *E. coli* causing PN and urosepsis in a child can also be found in the faeces of the same child. Therefore, the old theory – based on phenotypic markers of *E. coli* [16] – that the gut is the primary reservoir of these uropathogenic bacteria has

Table 1. Genetic clones of *E. coli* identified by PCR (*papG* classes I, II, III) and by PFGE(a–i) and O serotyping in 10 children with urosepsis

Origin of the isolate	Characteristics of the isolates from child no.									
	1	2	3	4	5	6	7	8	9	10
Blood	IIc O6	IId NT	IIIa O83	IId NT	IIIa O83	IIIf O6	IIg O1	IIIi O6	IIh O1	IIb O1
Urine	IIc O6	IId NT	IIIa O83	IId NT	IIIa O83	IIIf O6	IIg O1	IIIi O6	IIh O1	IIb O1
Faeces	IIc O6	IId NT	IIIa O83	IId NT	IIIa O83	II*	N†	IIIi O6	IIh O1	IIb O1

NT, O antigen non-typable.

*Growth on the first streaking area *papG* class II positive; five separate colonies *papG*-negative.

†No *papG* found.

been confirmed genetically. However, further studies on the pathogenesis and epidemiology of *E. coli* urosepsis are required before it is known why some individuals harbour pyelonephritogenic bacteria in their gut and how and for how long these bacteria colonise the gut and subsequently cause PN and occasionally sepsis.

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