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Virulence profiles in uropathogenic *Escherichia coli* isolated from pregnant women and children with urinary tract abnormalities

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ABSTRACT

Uropathogenic *Escherichia coli* is the leading etiologic agent of urinary tract infections, encompassing a highly heterogeneous group of strains. Although many putative urovirulence factors have been described, none of them appear in all uropathogenic *E. coli* strains, a fact that suggests that this group would be composed of different pathogenic subgroups. In this work, a study was performed on two collections of *E. coli* isolates proceeding from urine cultures from two groups of patients with urinary tract infection: pregnant women and children with urinary tract abnormalities. The isolates were analyzed for their virulence content and for their phylogeny by means of PCR determinations and of phenotypic assays. Associations among the virulence traits analyzed were searched for and this approach led to the identification of five urovirulence profiles. From a total of 230 isolates, 123 (53%) could be assigned to one of these profiles. A few loci appeared as markers of these profiles so that their presence allowed predicting the general virulence content of the strains. It is presumed that these conserved associations among the virulence functions would be devoted to ensure the coherence of the bacterial pathogenic strategy. In addition, three profiles appeared with significantly different frequencies depending on the host of origin of the isolates, indicating the existence of a correlation between the virulence content of the strains and their host specificity.

1. Introduction

Uropathogenic *Escherichia coli* (UPEC) is the leading etiologic agent of urinary tract infections (UTI), being responsible of as many as 80% of UTI in otherwise healthy people. UPEC belongs to the broader group of extraintestinal pathogenic *E. coli* (ExPEC), being highly related to other ExPEC strains that produce sepsis, neonatal meningitis, and also infections in animals such as colibacillosis in birds [1,2].

Several virulence-related functions have been described in UPEC strains such as certain fimbriae or afimbrial adhesins, cytotoxins, capsules and specific iron acquisition systems [3]. However, UPEC is far from being a homogeneous group of strains. Numerous studies focusing on their virulence content as well as on their phylogeny have revealed a great heterogeneity among them. Moreover, different UPEC strains may cause equal syndromes, a fact that has impeded the recognition of pathotypes [4–6]. From this view, it would be important to know whether conserved virulence profiles could be recognized in subsets of UPEC strains. This information could help to understand the pathogenic processes these strains carry out as well as their host spectrum.

The identification of new virulence factors could be helpful in the search for putative virulence profiles. In this sense, we have proposed that the production of higher-molecular-mass microcins (HMMM) would be related to urovirulence since these antibiotic activities are mainly produced by UPEC strains [7,8]. HMMM are a group of ribosomally-synthesized antibacterial peptides that includes four chromosome-encoded microcins (M, H47, I47, and E492) and a plasmid-encoded microcin, colicin V (ColV). They are all produced by strains of *E. coli* except for microcin E492, which is produced by strains of *Klebsiella pneumoniae* [9]. The genetic clusters for HMMM invariably contain a microcin activity gene, which encodes the antibacterial peptide backbone, and a cognate immunity gene. ColV is an unmodified peptide while chromosome-encoded microcins are post-translationally modified by the addition of a catechol group of the salmochelin type. Therefore, the genetic systems for these latter microcins also contain maturation genes whose products operate the post-translational modification

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of the microcin peptide precursor. Frequently, the genetic systems for chromosome-encoded microcins encode two or even three different microcin peptide precursors and contain a single set of ancillary genes devoted to microcin maturation and secretion. The most common co-productions are microcins H47 and M, and microcins H47 and I47. HMMM use the uptake pathway for catechol siderophores to enter susceptible cells, i.e. the catechol receptors and the TonB pathway. In *E. coli* K12, ColV uses the Cir receptor while chromosome-encoded microcins employ any catechol receptor, Cir, Fiu or FepA [9–12].

Studying UPEC isolates collected in a hospital of general assistance, the production of HMMM has been found to associate with several urovirulence traits [8]. Indeed, among isolates producing these microcins, different virulence-related contexts appeared following the type of microcin produced. Three groups of strains were described: (i) those co-producing microcins H47 and M, (ii) those producing microcin H47 only and (iii) those producing ColV. Strains of the first group are highly enriched in determinants for several virulence factors: S- and P-fimbriae, hemolysin, cytotoxic necrotizing factor, and the iron scavenging systems mediated by the siderophores salmochelins, yersiniabactin and aerobactin. The second group exhibits a particularly poor virulence context, and the third one contains determinants for iron uptake systems, often for P-fimbriae, and never for S-fimbriae or cytotoxins [8]. These results suggested the existence of different levels of compatibility among virulence functions that ranged from their obligatory coexistence to incompatibility. Considering that the isolates analyzed proceeded from a heterogeneous population, it was of interest to study UPEC strains from different human subpopulations in order to identify their characteristics.

In this work, we concentrated on the analysis of two collections of UPEC isolates coming from two distinct types of patients: pregnant women and children with urinary tract abnormalities. Associations among virulence-related traits were searched for with a special focus on their compatibility. In this instance, all isolates were analyzed whether they produced microcin activities or not. The aim was to identify urovirulence profiles and to assess their distribution between the two collections.

2. Materials and methods

2.1. Bacterial strains and growth conditions

Two collections of *E. coli* isolates from a Laboratory of Bacteriology of an obstetric and pediatric public hospital in Montevideo were examined. They proceeded from urine cultures indicated in the course of clinical diagnosis. One of the collections comprised 120 isolates coming from pregnant women who were in the course of a brief hospitalization for delivery or by threat of premature birth or abortion (PW collection). Following previously stated criteria [13] isolates included were: those proceeding from cultures with a colony count $>10^5$ cfu/ml (94 isolates) plus those which appeared with lower counts but with a record of symptomatic UTI (26 isolates). The 120 isolates proceeded from 107 women who were in the following gestational trimesters: one in the first, 28 in the second and 78 in the third. The other collection, of 126 *E. coli* isolates, proceeded from children (0–12 years-old) with functional or anatomical abnormalities of their urinary tract and who suffered from recurrent UTI (CH collection). They were ambulatory patients treated in a pediatric service of Nephrology for pathologies including vesicoureteral reflux, dysfunctional voiding and different types of congenital abnormalities. The 126 isolates came from 86 children and had been collected in 2008 and 2009 from cultures with a colony count $>10^5$ cfu/ml. For both collections, the sensitivity of the isolates to ampicillin (AMP), cefalotin (CEF), cefuroxime (CXM), nalidixic acid (NAL), ciprofloxacin (CIP) and trimethoprim–sulfamethoxazole (SXT) was determined by the disk diffusion method according to CLSI [14].

Besides these two collections, the studies also included the set of 27 microcin-producing UPEC isolates which had been previously presented and will be referred to as the reference collection. The archetypal *E. coli* strains CFT073 (co-producing microcins H47 and M) and H47 (producing microcin H47) were also analyzed [8]. Luria Bertani rich medium and minimal M63 medium supplemented with glucose were used [15].

2.2. Phenotypic assays

Microcin production and identification was assayed by patch test on minimal plates as previously described [8]. In brief, isolates producing antibacterial activities against a wild-type *E. coli* K12 strain were then tested on a collection of mutant strains deficient for microcin uptake, in order to recognize those activities employing the catechol uptake pathway. Then, these activities were identified following the immunity criterion by testing the corresponding isolates on a collection of recombinant *E. coli* K12 strains carrying the different immunity genes for HMMM.

The production of hemolysin was tested by stabbing the strains on tryptic soy agar plates with 5% sheep erythrocytes. After overnight incubation at 37 °C, strains were defined as hemolytic when they generated clear halos around the stabs.

2.3. Phylotyping and detection of virulence genes

Determinations were performed by PCR reactions, employing as template total genomic DNA extracted with the "Wizard genomic DNA purification system" (Promega).

E. coli strains were classified into the four main phylogenetic groups (A, B1, B2 and D) following a multiplex PCR procedure previously described [16].

The presence of a set of virulence-related loci was also assayed by PCR. The virulence genes surveyed were related to adhesion functions [type I fimbriae (*fimH*), P-fimbriae (*papC* and *papG* in its variants II and III), S-fimbriae (*sfaA*), and Dr/Afa adhesion/invasion (*draD*)]; toxins [hemolysin (*hlyA*), cytotoxic necrotizing factor (*cnf*), colibactin (*clbB*) and sat (*sat*)]; iron acquisition through siderophores [salmochelins (*iroBC* and *iroN* from the *iroA* locus), yersiniabactin (*fyuA*), aerobactin (*iutA*), and the Iha catechol receptor also involved in adhesion functions (*iha*)]; salmochelin production for catechol microcin synthesis in *E. coli* H47 (*iroBD*); the maturation gene for catechol microcins (*mchC*); activity genes for HMMM [M (*mcmA*), H47 (*mchB*), I47 (*mciA*), E492 (*mceA*) and ColV (*cvaC*)]; type II capsule (*kpsM*), and K1 capsule (*neuA* and *neuO*). Most primers used were already described [8]. Primers for *clbB* amplification were described by other authors [17] and those designed in this work are presented in Table 1. PCR determinations were carried out separately for each virulence loci. Amplifications were performed in a volume of 30 μl containing 1X buffer, 2 mM $MgCl_2$, 200 μM of each deoxynucleotide triphosphate, 500 nM of each primer and 1.25 U of Taq DNA polymerase (Invitrogen), plus 20–50 ng of template DNA. Conditions used were: 2 min at 94 °C, followed by 30 cycles of 94 °C for 30 s, the annealing temperature for 30 s and 72 °C for 30 s, and a final extension step at 72 °C for 2 min.

2.4. DNA sequencing

It was performed at the "Molecular Biology Unit" of the Pasteur Institute of Montevideo. PCR amplification products of microcin activity genes were purified using the MinElute PCR Purification Kit (Qiagen) in order to be sequenced. The amplicon for *mchB*