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Urinary tract infection in Uruguayan children: Aetiology, antimicrobial resistance and uropathogenic *Escherichia coli* virulotyping

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ABSTRACT

Uropathogenic *Escherichia coli* (UPEC) is the most frequent cause of urinary tract infection (UTI). Virulence factors (VFs) of UPEC in children are not well known. Circulating antibiotic resistance mechanisms in the community are increasing. In this study, the aetiological agents of UTI and antibiotic resistance mechanisms of 124 strains isolated from urine cultures from children with community-acquired UTI were determined. Virulotyping of isolated *E. coli* strains was also described. β-Lactam, fluoroquinolone and sulfonamide resistance genes as well as integrons were detected by PCR. *E. coli* phylogenetic groups and 25 VFs were sought by multiplex PCR. *E. coli* was the most frequent aetiological agent (88.7%), of which 48.2% belonged to phylogenetic group D and 35.5% to group B2. Moreover, 81.8% were considered UPEC and >93% had virulence structures, with *kpsMTII*, *fimH* and *iutA* being the most frequent. Most of the *E. coli* isolates were susceptible to amoxicillin/clavulanic acid (AMC) (87.3%), nitrofurantoin (97.3%), cefuroxime and third-generation cephalosporins (100%). Resistance levels to oxyimino-cephalosporins were higher in non-*E. coli* isolates, with circulation of integrons, $bla_{CTX\text{-}M\text{-}2}$ and $bla_{CMY\text{-}2}$ detected in the community. Moreover, 8.1% of isolates were resistant to fluoroquinolones, with *qnrB* found in two isolates. Resistance to trimethoprim/sulfamethoxazole was found in 37.9% of isolates, with 85.5% harbouring *sul* genes. *E. coli* isolated from children with UTI presented high rates of VFs. Nitrofurantoin, AMC and cefuroxime would be suitable antibiotics to treat UTI in children. However, the presence of integrons (fundamentally class 1) and circulation of broad-spectrum β-lactamases in the community makes continuous surveillance necessary.

1. Introduction

Urinary tract infection (UTI) is one of the most common bacterial infections and its occurrence in childhood is associated with underlying congenital anomalies, voiding dysfunction or vesicoureteral reflux. UTI may be associated with progressive kidney failure and renal dysplasia and is one of the most frequent causes of arterial hypertension in children [1,2]. Approximately 3% of prepubescent children are diagnosed with UTI, with it being more frequent in girls except during the first year of life [1,2].

E. coli is responsible for 70–80% of cases of UTI; other agents depend on specific regions and demographic characteristics of the patients, with *Klebsiella* spp. and *Proteus mirabilis* being frequently isolated [3].

The ability of *E. coli* to cause UTI depends, among other things, on the genetic content of each strain. Extraintestinal pathogenic *E. coli* and commensal *E. coli* differ in their phylogenetic group and virulence attributes [4].

Four main phylogenetic groups (A, B1, B2 and D) have been described in *E. coli* as defined by multilocus enzyme electrophoresis. Virulent extraintestinal *E. coli* strains belong mainly to groups B2 and D, whereas strains belonging to groups A and B1 are more probably commensals [5].

Uropathogenic *E. coli* (UPEC) clones possess different specialised virulence factors, including: adhesive structures composed of

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diverse adhesins (type 1 fimbriae, P fimbriae, S fimbriae and F1C fimbriae, Dr adhesins, and non-fimbrial adhesins); invasion-related structures such as polysaccharide capsule (group II and group III capsules), mainly K1 and K5, TraT (a protein related to resistance to serum bactericidal activity) and the *ibeA* gene (encoding an invasion protein involved in the development of meningitis in newborns); and iron acquisition structures (aerobactin and yersiniabactin). Secreted VFs such as α-haemolysin (HlyA) and cytotoxic necrotising factor (CNF1) are involved in pyelonephritis and kidney invasion, respectively [6].

Empirical treatment of UTI depends on the anatomical site of the infection (pyelonephritis or cystitis), the patient's age and whether it is for chemoprophylaxis (previous cystourethrography, recurrent UTI or in patients with upper grade of vesicoureteral reflux) [1].

In Uruguay, the principal antibiotics used in children are cefuroxime or cefuroxime axetil (when intravenous treatment is required or for oral treatment in children aged under 1 year, respectively), amoxicillin/clavulanic acid (AMC) or nitrofurantoin for non-complicated cystitis, and trimethoprim/sulfamethoxazole (SXT) for chemoprophylaxis (Normas Nacionales de diagnóstico, tratamiento y prevención de la Infección Urinaria en niños, in press).

Antibiotic susceptibility profiles for agents of UTI are variable and dynamic. Recently, an increase in the presence of strains with different resistance mechanisms has been reported in the community [7,8].

Whilst resistance to nitrofurantoin is primarily due to chromosomal mutations in the genes *nfsA* and *nfsB* [9], resistance to β-lactams and SXT is associated with the presence of enzymes that are usually coded by plasmids, thus being transferable. There are around a thousand variants of β-lactamases capable of hydrolysing different groups of β-lactams (http://www.lahey.org/studies/). SXT resistance is associated with the presence of *sul1*, *sul2* and *sul3* genes as well as dihydropteroate synthases and dihydrofolate reductases with lower affinity for sulfamethoxazole and trimethoprim, respectively [10].

In relation to quinolones, although resistance essentially involves mutations in the target site of action (*gyrA* and *parC*), plasmid-mediated quinolone resistance (PMQR) genes such as *aac(6′)-Ib-cr*, *qnrA* and *qnrB* have already been described in isolates from children in Uruguay [11].

In this study, we determined the aetiological agents involved in community-acquired UTI in Uruguayan children. In addition, antibiotic resistance mechanisms for the main antibiotics used were characterised, and virulotyping of *E. coli* strains was described. To the best of our knowledge, information related to resistance mechanisms and VFs in community-acquired uropathogens in children in Latin America is scarce. The results of this study could be helpful to improve local therapeutic guidelines.

2. Materials and methods

A descriptive study was conducted including 124 strains isolated from urine cultures obtained from children aged between 1 month and 14 years who consulted at the emergency service of Centro Hospitalario Pereira Rossell, the national referral children's hospital in Montevideo, Uruguay, from May–November 2010.

2.1. Microbiological identification and antibiotic susceptibility testing

Urine samples were obtained by catheterisation or mid-stream urine collection. Identification at species level was performed using a VITEK® 2 Compact System (bioMérieux, Marcy-l'Étoile, France). Antibiotic susceptibility testing and extended-spectrum β-lactamase (ESBL) screening were performed by disc diffusion tests and were interpreted following Clinical and Laboratory Standards Institute (CLSI) recommendations [12] and using the VITEK® 2 Compact System.

2.2. Detection of β-lactam, fluoroquinolone and sulfonamide resistance genes

Isolates resistant to ampicillin were further analysed by PCR for the presence of bla_{TEM}, bla_{SHV}, bla_{OXA-1} and bla_{OXA-2} using specific primers [13,14].

Enterobacterial isolates with a positive ESBL screening test underwent molecular confirmation by PCR using specific primers for ESBL genes frequently detected in Uruguay (bla_{CTX-M}, bla_{TEM}, bla_{PER-2} and bla_{SHV}) [13]. PCR products were fully sequenced on both strands. Plasmid *ampC* genes were detected by multiplex PCR according to previous reports [15].

Strains with a nalidixic acid minimum inhibitory concentration (MIC) of >2 μg/mL were further analysed by PCR and amplicon sequencing using primers for the genes *qnrA*, *qnrB* and *qnrS* [13].

The *sul1*, *sul2* and *sul3* genes were sought in sulfonamide-resistant isolates by PCR using primers previously described by Blahna et al. [10]. PCR for class 1 integrons was performed on those strains that harboured *sul1* genes, and PCR for class 2 integrons on those strains resistant to SXT [10].

Multiresistant isolates were defined as those having resistance to four or more classes of antibiotics [16]. To define different resistance patterns, we took into consideration the possible resistance mechanisms, hence resistance to ampicillin and cefalotin, including cefuroxime, were considered as the same profile.

2.3. Characterisation of phylogenetic groups and virulence factors in E. coli isolates

Four phylogenetic groups (A, B1, B2 and D) were sought by multiplex PCR according to Clermont et al. [5].

All isolates were also screened for five VFs according to Johnson et al. [6]: *papA* and *papC* (P fimbriae structural subunit and assembly); *sfa*/*foc* (S and F1C fimbriae); *afa*/*dra* (Dr binding adhesins); *iutA* (aerobactin receptor); and *kpsMTII* (group II capsules). Presence of two of the five markers defined the isolate as UPEC [6].

UPEC isolates were further analysed by multiplex PCR using specific primers for 20 additional VFs genes: adhesion proteins [*papEF*, *papG* (allele I, allele II, allele III and allele II/III), *sfaS*, *fimH*, *focG*, *nfaE*]; toxins (*hlyA*, *cnf*); siderophores (*fyuA*); capsule (*kpsMTIII*, *kpsMT* K1, *kpsMT* K5); 04 LPS (*rfc*); colicin V (*cvaC*); serum survival genes (*traT*); invasion of brain endothelium (*ibeA*); and a generic marker for uropathogenic pathogenicity-associated island (PAI) [17].

2.4. Statistical methods

Analysis of associations between phylogenetic group, VFs and antibiotic resistance were evaluated by χ^2 or Fisher's exact test. *P*-values of <0.05 were considered statistically significant. Statistical analyses were performed using SPSS Statistics for Windows v.17.0 software (SPSS Inc., Chicago, IL).

3. Results

3.1. Urinary tract infection aetiologies in children and antibiotic susceptibility profiles

In total, 124 isolates from urine cultures were studied. *E. coli* was the most frequent micro-organism (110 cases; 88.7%),

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