Extended-spectrum β-lactamases and plasmid-mediated quinolone resistance in enterobacterial clinical isolates in the paediatric hospital of Uruguay

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Objectives: To analyse the prevalence of resistance to β-lactams and plasmid-mediated quinolone resistance in Enterobacteriaceae in the paediatric hospital of Uruguay.

Methods: A total of 368 enterobacterial isolates collected between 1 May and 30 November 2009 were studied for the presence of extended-spectrum β-lactamases (ESBLs), qnr alleles and aac(6')Ib by phenotypic and molecular methods. The genomic context and transferability of β-lactamase and qnr genes were examined by PCR and conjugation, respectively.

Results: The proportion of inpatients having an infection caused by ESBL-producing enterobacteria was 0.23% (16/7073) in paediatrics wards, 0.64‰ (3/4696) in the neonatology department and 0.03‰ (1/32557) in the emergency department. ESBL-carrying enterobacteria constituted a total of 21.6% (16/74), 13% (3/23) and 0.37% (1/271) when samples were obtained from paediatrics wards, the neonatology department and the emergency department, respectively. Overall, CTX-M-2 (n=7), CTX-M-9 (n=3), CTX-M-15 (n=1), SHV-5 (n=5) and SHV-2 (n=2) β-lactamases were detected. Thirteen out of 20 ESBL-producing isolates also carried the aac(6')Ib gene, and the cr variant was detected in one of them. qnr alleles were detected in four isolates comprising two qnrA1 genes, a qnrB8-like variant and a new qnrB gene showing 26 amino acid differences from QnrB1.

Conclusions: The proportion of ESBL-producing enterobacteria in Uruguay’s paediatric hospital during the study period was 2.3 per 1000 hospitalized patients. The number of different microorganisms detected, as well as the various EBSLs, suggests the occurrence of sporadic episodes instead of nosocomial outbreaks. Nevertheless, the presence of new resistance genes reinforces the necessity for permanent surveillance programmes.

Keywords: antibiotic resistance, Enterobacteriaceae, integrons

Introduction

Enterobacteriaceae harbouring extended-spectrum β-lactamases (ESBLs) have been associated with an increase in mortality and healthcare-associated costs. Co-resistance to fluoroquinolones due to the dissemination of plasmid-mediated quinolone resistance (PMQR) associated with the classical (mutation-based) resistance mechanisms is frequent.

Although PMQR can be mediated by Qnr proteins (masking of target site), the production of Aac(6')Ib-cr or QepA and OqxAB efflux pumps, the first two mechanisms are by far the most frequent.

Data on the occurrence of both ESBLs and PMQR in paediatric patients from South America are scarce. So far, the only report on ESBLs from a paediatric population in Uruguay is that on PER-2 in typical enteropathogenic Escherichia coli (EPEC) strains isolated during the years 1991–93. Although PMQR has been reported in an adult population there are still no data concerning the paediatric population.
Materials and methods

A total of 368 enterobacterial isolates were recovered at the microbiology laboratory of Children’s Hospital Pereira Rossell (CHPR) between 1 May and 30 November 2009. Approximately 96% of these isolates were recovered from the following sources: urine culture (82.1%); blood samples (7.9%); faeces (3.8%); or surgical wounds (2.4%). Only one clinically relevant specimen per patient per hospitalization event was included. For re-hospitalized patients, data from different isolates were only recorded if they belonged to different species or to different resistance profiles. Identification to the species level was performed using the VITEK® 2 Compact system (bioMérieux, Marcy l’Étoile, France).

Antibiotic susceptibility tests were performed by a combination of diffusion tests (following CLSI recommendations) and using the VITEK® 2 Compact system. Additionally, MICs of cefotaxime, amikacin and ciprofloxacin were determined by Etest for those enterobacteria harbouring ESBLs, according to the manufacturer’s recommendations. ESBL screening and confirmatory tests were performed by disc diffusion, as suggested by CLSI guidelines, regardless of bacterial genus or species, as previously suggested for areas of high CTX-M enzyme prevalence. Isolates with positive ESBL screening results were further analysed by PCR for the presence of blaCTX-M, blaTEM, blaPER-2 and blaSHV genes using specific primers. Positive samples were re-amplified using Pfu DNA polymerase (Fermentas Life Sciences) and fully sequenced on both strands. The genes qnrA, qnrB, qnrS and aac(6’)-Ib and the cr variant were sought in ESBL-producing isolates by PCR and amplicon sequencing as previously described. We then used the deduced amino acid sequence of every available QnrB protein in public domain databases to construct a phylogenetic tree by the neighbour-joining method with the aid of MEGA4 software.

Isolates harbouring qnr alleles were also tested for the presence of insertion sequences such as ISCR1, IS26, IS903 and IS501 according to Eckert et al. All confirmed ESBL-producing isolates were analysed for the presence of class 1 integrons by PCR, using primers IS5/13, 5′/CS′/CS′, qacE1F/SuI1b and ORFend/F12R.

Conjugation assays were carried out using an E. coli J53 Rif′ strain as the recipient; transconjugants were selected on MacConkey agar plates (Oxoid) and fully sequenced on both strands. Incompatibility groups of plasmids carrying ESBL and/or AmpC were determined by PCR replication typing according to Carattoli et al.

Data on patients within the study period were obtained from the hospital’s information bureau (Sistema de Información Hospitalaria, El Centro Hospitalario Pereira Rossell en cifras 2009). Data on children in the neonatology service were obtained from the birth register of the CHPR.

Results

Two hundred and seventy-one enterobacteria were recovered from 32557 children in the emergency department, 234696 from the neonatology unit and 74/7073 from inpatients from different services of the CHPR (such as the paediatrics wards, intensive care unit, orthopaedics, haematology/oncology and the surgery department). A total of 4945/7073 inpatients (69.9%) were admitted from the emergency department.

Twenty enterobacterial isolates (20/368) were characterized as ESBL producers (16 from paediatrics wards, 3 from neonatology and 1 from the emergency department). Two different isolates were obtained from the same child in two different hospitalization events, yielding a CTX-M-2-producing E. coli strain and a CTX-M-8-producing Klebsiella pneumoniae strain.

The proportion of inpatients having at least one infection episode caused by ESBL-producing enterobacteria was 2.26% (16/7073) in paediatrics wards, 0.64% (3/4696) in the neonatology department and 0.03% (1/32557) in the emergency department. On the other hand the proportion of enterobacteria carrying ESBLs was 21.6% (16/74), 13% (3/23) and 0.37% (1/271) if samples were obtained from paediatrics wards, the neonatology unit and the emergency department, respectively.

ESBL-producing enterobacteria were recovered from 10 urine samples, 7 blood cultures, 1 skin lesion sample, 1 catheter tip sample and 1 synovial fluid sample. ESBL genes are shown in Table 1.

Thirteen out of 20 ESBL-harbouring isolates also carried the gene aac(6)′Ib-coupled to blaCTX-M-2, blaCTX-M-8, blaCTX-M-9, blaCTX-M-15, blaSHV-2 or blaSHV-5. Of these, one isolate carried the aac(6)′Ib-variant in a class 1 integron and displayed an MIC of amikacin as low as 3 mg/L, whereas another harboured the aac(6)′Ib-cr variant along with blaCTX-M-15.

Four isolates harboured qnr variants. Two Enterobacter cloacae isolates carried the genes qnrA1-ampr linked to ISCR1, one Citrobacter freundii carried a qnrB-like variant along with blaCTX-M-2, and one K. pneumoniae harboured blaCTX-M-8 and a qnrB variant linked to IS501. Regarding this isolate, both determinants were simultaneously transferred by conjugation, and transconjugants (TcKp737) showed an approximate 10-fold increase in ciprofloxacin MIC (0.38 mg/L versus 0.032 mg/L for the rifampicin-resistant E. coli receptor strain).

The partial nucleotide sequence of the qnrB variant (606 bp), obtained with primers qnrB-F and qnrB-R linked to IS501, showed 77% similarity with qnrB17, whereas the deduced amino acid sequence showed 87% identity with the corresponding protein, displaying 26 differences with QnrB1, 25 of which have not been described in the http://www.lahey.org/qnrStudies web site.

Class 1 integrons were detected in 14/20 strains, displaying eight distinct genetic arrays (Table 1). Such arrays carried 11 different gene cassettes, partially explaining resistance to β-lactams (Table 1). The proportion of ESBL-producing enterobacteria in the paediatrics ward was 2.26% (16/7073) in paediatrics wards, 0.64% (3/4696) in the neonatology department and 0.03% (1/32557) in the emergency department. On the other hand the proportion of enterobacteria carrying ESBLs was 21.6% (16/74), 13% (3/23) and 0.37% (1/271) if samples were obtained from paediatrics wards, the neonatology unit and the emergency department, respectively.

ESBL-producing enterobacteria were recovered from 10 urine samples, 7 blood cultures, 1 skin lesion sample, 1 catheter tip sample and 1 synovial fluid sample. ESBL genes are shown in Table 1.

Discussion

The proportion of ESBL-producing enterobacteria in the paediatrics wards of the CHPR during the study period was 2.26% (16/7073) in paediatrics wards, 0.64% (3/4696) in the neonatology department and 0.03% (1/32557) in the emergency department. On the other hand the proportion of enterobacteria carrying ESBLs was 21.6% (16/74), 13% (3/23) and 0.37% (1/271) if samples were obtained from paediatrics wards, the neonatology unit and the emergency department, respectively.

ESBL-producing enterobacteria were recovered from 10 urine samples, 7 blood cultures, 1 skin lesion sample, 1 catheter tip sample and 1 synovial fluid sample. ESBL genes are shown in Table 1.

Conjugation assay and replicon typing results of the 20 ESBL-harbouring isolates are shown in Table 1.

The partial nucleotide sequence of the qnrB variant (606 bp), obtained with primers qnrB-R and tspAISECp1, showed 77% similarity with qnrB17, whereas the deduced amino acid sequence showed 87% identity with the corresponding protein, displaying 26 differences with QnrB1, 25 of which have not been described in the http://www.lahey.org/qnrStudies web site.

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ESBL-producing enterobacteria were recovered from 10 urine samples, 7 blood cultures, 1 skin lesion sample, 1 catheter tip sample and 1 synovial fluid sample. ESBL genes are shown in Table 1.

Conjugation assay and replicon typing results of the 20 ESBL-harbouring isolates are shown in Table 1.
Table 1. Main features of the ESBL-producing enterobacteria isolated in this study

<table>
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<th>Number</th>
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<th>CAZ</th>
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<th>GEN</th>
<th>NAL</th>
<th>CIP</th>
<th>SXT</th>
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<th>ESBL</th>
<th>Scs-3s</th>
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MIC (mg/L)

- T2P: piperacillin/tazobactam
- CTX: cefotaxime
- CAZ: ceftazidime
- AMK: amikacin
- GEN: gentamicin
- NAL: nalidixic acid
- CIP: ciprofloxacin
- SXT: trimethoprim/sulfamethoxazole
- ICU: intensive care unit
- HO: haematology/oncology
- 5cs-3cs: variable region of class 1 integron (slashes separate different integrons)
- Acid: plasmid incompatibility group
- Tc: conjugant

The table above summarizes the main features of the ESBL-producing enterobacteria isolated in this study. The MIC values for various antibiotics are presented for each isolate. The table includes the number, service, isolation date, sample type, strain, and resistance profile for each isolate. The MIC values are shown as the highest concentration of antibiotic that inhibits visible growth. The table also includes information on the presence of PMQR genes, which are associated with resistance to fluoroquinolones. The presence of PMQR genes can be identified by the presence of a specific mutation in the qnr gene. The table shows that many of the isolates were resistant to multiple antibiotics, including ESBLs and PMQR genes, highlighting the need for appropriate antibiotic therapy and infection control measures. The results also suggest the potential for antibiotic resistance in hospitalized children in Uruguay, highlighting the importance of continued monitoring and surveillance of antibiotic resistance patterns.
The phylogenetic analysis indicates that it is clearly different from the rest of the previously described QnrB proteins (see Figure 1). Additionally, this is the first description of a qnrB allele linked to IS\textsubscript{Ecp1}. This insertion sequence has been found next to several antibiotic resistance genes, such as rmtC (which confers resistance to aminoglycosides), and to various \(\beta\)-lactamases, mainly CTX-M-15.

Since the occurrence of ESBL-producing enterobacteria in the CHPR apparently is not associated with outbreaks, the clinical details of patients harbouring such microorganisms should be studied to identify any predisposing factor that may account for infections caused by them. Nevertheless, this work represents a starting point for the development of surveillance programmes aimed at the detection of ESBLs and PMQR.
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**Transparency declarations**
None to declare.

**References**