Esbl (Extended Spectrum Beta Lactamase) Infections - An Emerging Problem in Children

Petrit Biberaj
University "Aleksandër Xhuvani" Elbasan

Indrit Bimi
University "Aleksandër Moisiu" Durrës

Daniela Bimi
Maternity "Queen Geraldina" Tirana

Background and aims

There is an emergence of infections with ESBL-producing micro-organisms recently. They remain poorly characterised in children. We sought to characterise children in a teaching hospital from whom an ESBL-producing organism had been isolated.

Methods

We identified all children under sixteen years of age who had ESBL-producing organism isolated in the year 2016 in children admitted to pediatric hospital at University Hospital centre "Mother Theresa", Tirana. The referral source was contacted for further information where possible. Clinical details and treatment were analysed to determine likelihood of infection or colonisation.

Results

Fifteen children were identified, aged three months to nine years. Thirteen isolates were obtained from urine, one each from a wound swab and a cough swab. Three isolates were obtained from hospitalised children, but only one of these needed treatment. This child presented with lymphadenitis and continued to be pyrexial and irritable despite treatment with cephalosporin. Subsequently ESBL producing organism was isolated from the urine and she responded to appropriate antibiotic. Data was available for nine out of twelve patients originating from the community. Of these, four had repeat specimens which didn't grow ESBL-producer again. Three were treated and two remained asymptomatic and well.

Conclusions: ESBL-producing organisms are increasingly isolated in children, but from our review appear frequently to be colonisers rather than pathogens. However, it is very important to be vigilant about the possibility of ESBL infection in children especially if they fail standard antibiotic therapy.

Introduction

Resistance of Gram-negative bacteria to antibiotics has increased at an alarming pace over the last two decades, particularly the emergence of Enterobacteriaceae resistant to third-generation cephalosporins and aztreonam1 which is commonly associated with the expression of extended-spectrum beta-lactamases (ESBLs) (1). These enzymes confer resis ance to nearly all beta-lactam antibiotics such as ceftazidime, cefotaxime, ceftriaxone, monobactam – aztreonam, and related oxyimino beta-lactams (2). If Enterobacteriaceae are resistant to one of the extended-spectrum cephalosporins, it means they are therapeutically resistant to all the cephalosporins even though antimicrobial sensitivity is indicated in the laboratory test results (3). Moreover, many ESBL-producing Enterobacteriaceae are also resistant to other antimicrobial agents such as aminoglycosides, trimethoprim, and the quinolones which poses a serious antibiotic management problem as the genes for ESBL production are easily transferred through plasmids (4). ESBL-producing Enterobacteriaceae have worldwide distributions with varying degree of prevalence in community as well as hospitals (5). Nowadays, infections due to ESBL-producing Enterobacteriaceae are concerning for many reasons including increased hospital costs, length of stay, and mortality rates (6). For the pediatric population, blood stream infections and urinary tract infections (UTIs) due to Enterobacteriaceae resistant to ESBL are an emerging problem.1 This alerts clinical microbiologists to identify these ESBL-producing organisms parallel to

antimicrobial susceptibility testing even in resource-limited settings by applying simple screening and confirmatory methods. Data obtained from such methods are so valuable to develop appropriate institutional-based drug therapy guieline (7). Though various phenotypic ESBL detection methods have been described, implementation of highly sensitive and specific methods in resource-limited areas is challenging yet. Infections caused by ESBL- or plasmid-mediated AmpC-producing Enterobacteriaceae are often treated by carbapenems (e.g., ertapenem, imipenem, meropenem, and doripenem) which are antimicrobials of last resort and crucial for the management of life-threatening health care-associated infections. However, due to recent emergence and spread of imipenem/meropenem-resistant Enterobacteriaceae throughout the world, clinical utility of this group of antibiotics is under threat. Production of carbapenemases that are capable of hydrolyzing the carbapenems and loss of outer membrane proteins are major mechanisms through which Enterobacteriaceae develop resistance against this group of drugs (8). The aim of the study was to estimate the frequency of ESBL-producing organism.

Materials and methods

We identified all children under sixteen years of age who had ESBL-producing organism isolated in the year 2016 in children admitted to pediatric hospital at University Hospital centre "Mother Theresa", Tirana. The referral source was contacted for further information where possible. Clinical details and treatment were analysed to determine likelihood of infection or colonisation. Demographic characteristics of the patients were recorded using predesigned sheets after obtaining informed consent. From the UTIs-suspected children, first morning mid-stream urine samples were collected using sterile wide-mouth container. The study participants' parents/guardians were given appropriate instructions before providing urine samples. Urine specimens immediately after collection were brought to microbiology laboratory for bacterial analysis.

Culture and identification

Enterobacteriaceae were classified to species levels using triple sugar iron, indole, citrate, urea, lysine decarboxylase, and motility. After identification, each Enterobacteriaceae was subjected to ESBL and carbapenemase detections as per Clinical and Laboratory Standards Institute (CLSI) and European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines.11,12

Drug susceptibility patterns

The disk diffusion was performed, and after 16–18 hours of incubation at 37°C, zone of inhibition was measured and interpreted as recommended by the CLSI.11 Using a sterile wire loop, three to five pure colonies were picked from MacConkey agar and emulsified in nutrient broth. Standard inoculums adjusted to 0.5 McFarland using McFarland Densitometer were swabbed onto Muller-Hinton agar (dispensed on 100 mm plate). Drug susceptibility testing of all Enterobacteriaceae was performed using disk diffusion method against amoxicillin (30 µg, BD), amoxicillin-clavulanic acid (30 µg, BD), chloramphenicol (30 µg, BD), gentamicin (10 µg, BD), sulfamethoxazole-trimethoprim (1.25 µg, BD), cefotaxime (30 µg, BD), cefoxitin (30 µg, Oxoid), tetracycline (30 µg, BD), nitrofurantoin (300 µg, BD), norfloxacin (5 µg, BD), imipenem (10 µg, Oxoid), and meropenem (10 µg, Oxoid). In this study, multidrug resistance was defined as simultaneous resistance to two or more drugs of different classes of antimicrobial agent.

ESBL detection

Initial screening of Enterobacteriaceae for ESBL was done based on diameters of zone of inhibitions produced by ceftazidime (30 µg, BD), ceftriaxone (30 µg, BD), and cefotaxime (30 µg, BD) according to the CLSI screening criteria. The breakpoints indicative of suspicion for ESBL production were ≤22 mm for ceftazidime, ≤25 mm for ceftriaxone, and ≤27 mm for cefotaxime. A combined disk method was used as a confirmatory phenotypic method for ESBLs detection according to CLSI. Ceftazidime (30 µg, BD) and cefotaxime (30 µg, BD) disks alone and their combinations with clavulanic acid (30 µg/10 µg) were used for phenotypic confirmations of ESBLs presence. A ≥5 mm increase in zone diameters for either of the cephalosporin disks or their respective cephalosporin/clavulanate disks was interpreted as ESBL producer. Double-disk synergy method was compared against combination disk method for detection of ESBL to know if it was the best suitable phenotypic method in resource-limited routine bacteriology laboratory. The antibiotic disks used were ceftriaxone (30 µg, BD), cefotaxime (30 µg, BD), ceftazidime (30 µg, BD), aztreonam (30 µg, BD), and amoxicillin/clavulanic acid (20/10 µg, BD) according to EUCAST.12 The four antibiotics were placed at distances of 20 mm edge to edge from the amoxicillin/clavulanic acid disk that was placed in the middle of the plate. After 24 hours of incubation, if an enhanced zone of inhibition between either of the cephalosporin antibiotics and the amoxicillin/clavulanic acid disk occurred, the test was considered as ESBL positive.

Results and Discussion

Fifteen children were identified, aged three months to nine years. Thirteen isolates were obtained from urine, one each from a wound swab and a cough swab. Three isolates were obtained from hospitalised children, but only one of these needed treatment. This child presented with lymphadenitis and continued to be pyrexial and irritable despite treatment with cephalosporin.

Enterobacteriaceae were isolated from urine specimens. Majority of Enterobacteriaceae (69.2%, n=9/13) were isolated from urine cultures. The most frequent isolates were K. pneumoniae (30.8%) and E. coli (23.1%).

Other Enterobacteriaceae isolates were Morganella morganii and Enterobacter aerogenes. All Enterobacteriaceae showed the highest resistance to amoxicillin (89.1%), sulfamethoxazole-trimethoprim (83.6%), and cefotaxime (85.5%), and least resistance to nitrofurantoin (36.9%), imipenem (12.2%), and meropenem (14.6%). The frequent isolates K. pneumoniae (57.57%, n=19/33) showed the highest resistance to cefotaxime(100%), amoxicillin (94.7%), amoxicillin-clavulanic acid (89.5%), sulfamethoxazole-trimethoprim (89.5%), and gentamicin (89.5%). They showed the lowest resistance to imipenem (10.5%), meropenem (15%), and norfloxacin (15.8%) compared to the other tested drugs. In our study, cefotaxime was least effective (100% resistance) against K. pneumoniae (57%,), M. morganii.

Subsequently ESBL producing organism was isolated from the urine and she responded to appropriate antibiotic. Data was available for nine out of twelve patients originating from the community.

Of these, four had repeat specimens which didn't grow ESBL-producer again. Three were treated and two remained asymptomatic and well.

Inappropriate and incorrect administration of antimicrobial agents in empirical therapies, lack of appropriate infection-control strategies which can cause a shift to increase in prevalence of resistant organisms in the community, and the selective pressure created for the use of third-generation cephalosporins have been described as most important factors in the appearance of ESBL-producing strains, though many reasons can be responsible and mentioned (9).

All ESBL-positive *Enterobacteriaceae* showed the highest resistance to amoxicillin (89.1%), sulfamethoxazole-trimethoprim (83.5%), and cefotaxime (85.5%). It means that the use of these antibiotics for the treatment of infection caused by ESBL-producing strains may result in failure in significant proportion of cases. The choice of antibiotic agents effective against ESBLs-producing species is currently limited, which may cause serious therapeutic problems in the future (10). In our study, the organisms were only susceptible to norfloxacin (63.9%) and cefoxitin (50%) compared to other commonly tested drugs.

Conclusion

The increasing frequency of ESBL-producing Enterobacteriaceae among children is an important problem for both microbiologists and clinicians. In resource-limited settings, double-disk synergy method can be implemented for screening and confirming ESBL production that might give valuable information for appropriate antibiotics selection and controlling the spread of ESBL-positive Enterobacteriaceae. However, it is very important to be vigilant about the possibility of ESBL infection in children especially if they fail standard antibiotic therapy.

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