Short Communication

Prevalence and antibiotic resistance profiles of extended spectrum β-lactamase-producing Escherichia coli among paediatric patients with urinary tract infection in St. Patricks’ Hospital, Mile Four, Abakaliki, Ebonyi State, Nigeria

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Abstract:

Background: The extended spectrum beta-lactamase (ESBL)-producing Escherichia coli strains which have been implicated in septicaemia among hospitalized children is a serious concern due to their high resistance rates to commonly used antimicrobial agents. The objective of this study was to determine the prevalence and antibiotic susceptibility of urinary ESBL-producing E. coli in paediatric patients who had clinical evidence of urinary tract infections (UTI).

Methodology: Clean catch specimens of urine collected from 100 eligible paediatric patients with clinical evidence of UTI in St. Patricks’ Hospital, Mile Four, Abakaliki, Ebonyi State, were cultured for isolation of E. coli using standard bacteriological techniques. Isolates were confirmed for ESBL production by double disk synergy test (DDST), and antibiotic susceptibility of the ESBL-producing ones was determined by the modified Kirby Bauer disk diffusion method.

Results: Twenty one (21%) E. coli were isolated out of which 11 (52 %) were ESBL producers, all of which were totally resistant (100%) to cefotaxime, ticarcillin and sulfamethoxazole-trimethoprim, 85% to aztreonam and 83% to ceftazidime. The multiple antibiotic resistance index (MARI) values ranged from 0.4 to 0.9, which implies high usage of antimicrobials.

Conclusion: The high prevalence of multi-drug resistant ESBL-producing E. coli obtained in this study shows that there has been overuse (abuse or misuse) of antibiotics in the study area. There is need for antimicrobial stewardship programme that will ensure prudent use of antimicrobial agents to forestall the emergence and spread of multi-drug resistant bacteria.

Keywords: Paediatrics, Escherichia coli, ESBL, urine, multi-drug resistance

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Prochains de prévalence et de résistance aux antibiotiques d’Escherichia coli produisant des β-lactamases à spectre étendu chez des patients pédiatiques présentant une infection des voies urinaires à l’hôpital St. Patricks, Mile Four, Abakaliki, État d’Ébonyi, Nigéria

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Abstrait:

Contexte: Les souches d’*Escherichia coli* productrices de bêta-lactamase à spectre étendu (BLSE) qui ont été impliquées dans la septicémie chez les enfants hospitalisés constituent un grave problème en raison de leur taux de résistance élevé aux agents antimicrobiens couramment utilisés. L’objectif de cette étude était de déterminer la prévalence et la sensibilité aux antibiotiques d’*E. coli* producteurs de BLSE dans l’urine chez les patients pédiatriques présentant des signes cliniques d’infections des voies urinaires (UTI).

Méthodologie: Des échantillons d’urine prélevés chez 100 patients pédiatriques éligibles présentant des signes cliniques d’UTI à l’hôpital St. Patrick’s, à Mile Four, à Abakaliki, dans l’État d’Ebonyi, ont été cultivés pour l’isolement de *E. coli* à l’aide de techniques bactériologiques classiques. Les isolats ont été confirmés pour la production de BLSE par un test de synergie à double disque (DDST) et la sensibilité aux antibiotiques des producteurs de BLSE a été déterminée par la méthode de diffusion sur disque de Kirby Bauer modifiée.

Résultats: Vingt et un (21%) *E. coli* ont été isolés, dont 11 (52%) étaient des producteurs de BLSE, qui étaient tous totalement résistants (100%) au céfotaxime, à la ticarcilline et au sulfaméthoxazole-triméthoprim, 85% à l’aztréonam et au 83 % en ceftazidime. Les valeurs de l’indice de résistance multiple aux antibiotiques (MARI) allaient de 0,4 à 0,9, ce qui implique une utilisation élevée d’antimicrobiens.

Conclusion: La prévalence élevée d’*E. coli* productrice de BLSE résistante à plusieurs médicaments obtenue dans cette étude montre qu’il y a eu surutilisation (abus ou abus.) d’antibiotiques dans la zone d’étude. Un programme de gestion des antimicrobiens est nécessaire pour garantir une utilisation prudente des agents antimicrobiens afin de prévenir l’émergence et la propagation de bactéries multirésistantes aux médicaments.

Mots-clés: pédiatrie, *Escherichia coli*, BLSE, urine, multirésistance

Introduction:

Beta-lactam antibiotics are the most commonly used therapeutic agents, accounting for over 50% of antibiotics used for treatment of bacterial infections, due to their broad antibacterial spectrum and excellent safety profile (1). The use of β-lactam antibiotics had however been hampered by β-lactamases which are enzymes that inactivate the β-lactam ring of the antibiotics thereby rendering them ineffective. The extended spectrum β-lactamases (ESBLs) produced by Gram-negative bacilli such as *Escherichia coli* have the ability to hydrolyse β-lactams including the third generation cephalosporins and aztreonam and yet are inhibited by clavulanic acid. ESBLs are mostly plasmid-mediated and can be transferred from one strain to another among bacterial species.

The prevalence of antibiotic resistance in bacterial species such as *E. coli*, *Klebsiella* and *Proteus* species isolated from clinical patients is increasing worldwide (2). In particular, strains of *E. coli* and *Klebsiella* species producing ESBL enzymes have become a worldwide problem with serious negative impact on the efficacy of β-lactam therapy. Thus, there is need for efficient infection control practices for containment of outbreaks of these strains.

The presence of ESBLs is associated with multi-drug resistance to various other classes of antibiotics such as monobactam, carbapenem, amino- glycosides, ciprofloxacin and erythromycin (1). The detection of these enzymes is therefore important in preventing treatment failures from infections caused by these pathogens. The objective of this study therefore is to determine the prevalence and antibiotic resistance patterns of urinary ESBL-producing *E. coli* isolates from paediatric patients in St. Patrick’s Hospital, Mile Four, Abakaliki, Ebonyi State.

Materials and methods:

Study area

St. Patrick’s hospital, Mile Four, Abakaliki is a missionary hospital located in Ebonyi State. The hospital specializes majorly in maternal and child care, and has an outpatient department (OPD), nursery ward, children’s ward, maternity ward, and child’s welfare clinic.

Study population

The study population consists of children (15 years and below) with clinical evidence of UTI who were seen at the outpatient department (OPD), and those hospitalized in the nursery and children’s wards.

Ethical clearance

Ethical clearance was obtained from the Research and Ethics committee of the hospital before the commencement of sample collection. Informed consent was also obtained from the parents/guardians of the children.

Sample collection, culture isolation and biochemical identification

Urine sample was collected as clean-catch early morning urine from each child into a sterile container free of preservatives. Each
specimen was labelled accordingly with the date and time of collection, and identification number. Samples were transported immediately to the Laboratory unit of the Department of Applied Microbiology, Ebonyi State University, for bacteriological analysis.

The urine samples were cultured on eosin methylene blue (EMB) and MacConkey agar plates, and incubated aerobically at 37°C for 24 hours. E. coli was presumptively identified as green metallic sheen colonies on EMB agar and as non-mucoid pinkish colonies on MacConkey agar. The isolates were confirmed as E. coli using standard conventional biochemical identification tests scheme (3).

**Antibiotic susceptibility test of isolates**

Antimicrobial susceptibility test of the isolates was done using the modified Kirby-Bauer disk diffusion technique (4) on Mueller-Hinton (MH) agar. Inoculum of each isolate prepared by suspending pure colonies of the bacteria from nutrient agar subculture plate in normal saline was standardized by comparing with 0.5 McFarland turbidity standards. This was inoculated on the surface of the MH agar plates with sterile swab using the following antibiotic disks (Oxoid, UK); cefotaxime (CTX, 30μg), ceftazidime (CAZ, 30μg), tobramycin (TOB, 15μg), aztreonam (AZ, 30 μg), gentamicin (GEN, 10μg), sulfamethoxazole-trimethoprim (SXT, 75μg), ciprofloxacin (CIP, 5μg), ertapenem (ERT, 10μg), ticarcillin (TIC, 75μg), amikacin (AMK, 30μg), and amoxicillin-clavulanic acid (AMC, 25μg/10 μg).

The plates were incubated aerobically at 37°C for 18-24 hours. The zones of inhibition were measured with a meter rule and the results interpreted according to the Clinical and Laboratory Standards Institute guidelines (5).

**Phenotypic confirmatory test for ESBL production**

*Escherichia coli* isolates exhibiting reduced susceptibility to any of the 3rd generation cephalosporins (suspected ESBL producers) were phenotypically confirmed for ESBL production using the double disc synergy test (DDST) (6). This was performed as a standard disc diffusion assay on Mueller-Hinton agar (Oxoid, UK) plates in line with CLSI guideline (5). Sterile swabs were dipped into standard bacterial suspension standardized to 0.5 McFarland turbidity standards and inoculated on the MH agar plates. Amoxicillin/clavulanic acid (20/10 μg) disk was placed at the centre of the plate and cefotaxime (30μg) and ceftazidime (30μg) were placed at a distance of 15 mm (centre to centre) from the central disk.

The plates were incubated aerobically at 37°C for 24 hours. ESBL production was confirmed when the zones of inhibition of the cephalosporins (ceftaxime and ceftazidime) increased in the presence of amoxicillin-clavulanic acid disk. A ≥ 5mm increase in the inhibition zone diameter for either of the cephalosporins tested in combination with amoxicillin-clavulanic acid over the zone when tested alone, confirmed ESBL production (6). *E. coli* ATCC 25922 was used as quality control strain.

**Determination of multiple antibiotic resistance index (MARI)**

Multiple antibiotic resistance index (MDRI) was calculated as MDRI = a/b where ‘a’ is the number of antibiotics to which the isolate was resistant to, while ‘b’ is the total number of antibiotics to which the isolate was tested against (7).

**Results:**

Twenty one *E. coli* were isolated from urine samples of 100 children studied; 9 isolates were from those in age group < 5 years, 4 (44.4%) of which were ESBL producing; 4 isolates were from those in age group 5-10 years, 2 (50%) of which were ESBL producer; and 8 isolates were from age group 11-15 years, 5 (62.5%) of which were ESBL producers ($X^2 = 0.1740, p = 0.9167$) (Table 1).

ESBL-producing *E. coli* isolates were totally resistant to cefotaxime, aztreonam, ticarcillin, sulfamethoxazole-trimethoprim and ciprofloxacin among children aged <5 years. Isolates also exhibited total resistance to cefotaxime, ceftazidime, ticarcillin and sulfamethoxazole-trimethoprim among children aged 5-10 years, and similarly, there was total resistance of isolates to cefotaxime, aztreonam, ticarcillin and sulfamethoxazole-trimethoprim from children aged 11-15 years (Table 2)
Table 1: Frequency of *Escherichia coli* and ESBL producing isolates from urine samples of paediatrics patients in St Patricks Hospital, Mile Four, Abakaliki, Ebonyi State, Nigeria

| Age group (years) | No of urine samples | No of *Escherichia coli* isolates (%) | No of ESBL-producing *E. coli* isolates (%) |
|-------------------|---------------------|--------------------------------------|------------------------------------------|
| < 5               | 34                  | 9 (26.5)                             | 4 (44.4)                                 |
| 5 - 10            | 39                  | 4 (10.3)                             | 2 (50.0)                                 |
| 11 - 15           | 27                  | 8 (29.6)                             | 5 (62.5)                                 |
| Total             | 100                 | 21 (21.0)                            | 11 (52.4)                                |

X² = 0.1740, p = 0.9167 (no significance difference)

Table 2: Antibiotic susceptibility of ESBL-producing *Escherichia coli* urinary isolates from children at St. Patricks Hospital, Abakaliki, Mile Four, Ebonyi State, Nigeria

| Age group (years)/ Antibiotics(μg) | < 5 (n = 4) | 5 - 10 (n = 2) | 11 - 15 (n = 5) |
|-----------------------------------|------------|---------------|-----------------|
| Cefotaxime (30)                   | Susceptible | Resistance    | Susceptible     | Resistance     | Susceptible | Resistance     |
|                                  | 0          | 4             | 0               | 2              | 0           | 5              |
| Ceftazidime (30)                  | 1          | 3             | 0               | 2              | 1           | 4              |
| Aztreonam (30)                    | 0          | 4             | 1               | 1              | 0           | 5              |
| Amikacin (30)                     | 2          | 2             | 2               | 0              | 4           | 1              |
| Ticarcillin (75)                  | 0          | 4             | 0               | 2              | 0           | 5              |
| Ertapenem (10)                    | 1          | 3             | 1               | 1              | 2           | 3              |
| Gentamicin (10)                   | 1          | 3             | 1               | 1              | 4           | 1              |
| Sulfamethoxazole-trimethoprim (75)| 0          | 4             | 0               | 2              | 0           | 5              |
| Ciprofloxacin (5)                 | 0          | 4             | 1               | 1              | 1           | 4              |
| Tobramycin (15)                   | 1          | 3             | 1               | 1              | 2           | 3              |

n = no of *Escherichia coli* isolates

Table 3: Multiple antibiotic resistance index (MARI) of the ESBL-producing *Escherichia coli* isolates from paediatric patients in St Patrick Hospital, Abakaliki, Ebonyi State, Nigeria

| ESBL producing *Escherichia coli* isolate | MARI |
|------------------------------------------|------|
| < 5 years                                 |      |
| E7                                       | 1.0  |
| E10                                      | 0.7  |
| E14                                      | 0.9  |
| E20                                      | 0.8  |
| Average MARI = 0.9                       |      |
| 5 - 10 years                             |      |
| E17                                      | 0.9  |
| E18                                      | 0.4  |
| Average MARI = 0.7                       |      |
| 11 - 15 years                            |      |
| E2                                       | 0.8  |
| E4                                       | 0.7  |
| E11                                      | 0.7  |
| E13                                      | 0.6  |
| E21                                      | 0.8  |
| Average MARI = 0.7                       |      |

The average multiple antibiotic resistance index (MARI) values of the ESBL-producing *E. coli* isolates in age groups < 5 years, 5-10 years and 11-15 years are respectively 0.9, 0.7, and 0.7 (Table 3). There was no statistically significant difference in the average MARI values between the age groups (p > 0.05)

**Discussion:**

In our study, the prevalence of *E. coli* in 100 children with urinary tract infection at St. Patricks Hospital, Abakaliki, Ebonyi State, Nigeria is 21%. In a related study by Sani et al., (8), the prevalence of 45% for *E. coli*
isolates was reported in 222 urinary samples, and in another similar one by Sabrina et al., (9), a prevalence of 37.5% for E. coli was reported in 280 urinary samples of children with urinary tract infection. Although these studies are similar to the present one conducted by us, the prevalence of E. coli in urinary tract infection in our study is lower than the prevalence in these other studies, probably because of the smaller sample size in our study.

In our study, 52% (11 of 21) of the E. coli isolates were phenotypically confirmed ESBL-producer, which compared with a related study by Amita and Rajesh who reported 58% E. coli to be ESBL producer in their study (10). In the study by Babak et al., (11), a high prevalence of ESBL producer in E. coli of 68.2% in children aged 9-12 years was reported, which agrees with 62.5% reported among comparative age group (11-15 years) in our study. This may suggests a higher prevalence of ESBL producers among urinary E. coli isolates in older children.

The ESBL-producing E. coli isolates in our study exhibited total resistance to cefotaxime, ticarcillin and sulfamethoxazole/trimethoprim among the children across age strata, and to aztreonam, ceftazidime and ciprofloxacin in specific age groups. This is similar to the study of Rajesh et al., (12) who reported high resistance rates of EBSL-producing E. coli isolates to ticarcillin, ampicillin, monobactam (aztreonam) and cephalosporins. The average multiple antibiotic resistance index (MARI) value of 0.8 reported for the ESBL-producing E. coli isolates in this study is an indication that antibiotics are overused in our environment.

Conclusion:

The high prevalence of multi-drug resistant ESBL-producing E. coli obtained from children with urinary tract infection in St. Patrick Hospital, Abakaliki, Ebonyi State, shows that there has been overuse (abuse or misuse) of antibiotics in the area. There is need for antimicrobial stewardship programme that will ensure prudent use of antimicrobial agents to forestall the emergence and spread of multi-drug resistant bacteria.

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