Effectiveness of temocillin on extended-spectrum beta-lactamase producing *Escherichia coli* isolates from patients attending the Niamey General Reference Hospital

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Extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* urinary tract infections represent a major global health problem, constituting a public health emergency with high patient morbidity and mortality. Temocillin serves as an alternative antibiotic for treating these infections and conserving carbapenems. A total of 209 isolates of uropathogenic *Escherichia coli* were collected in the Microbiology Laboratory of Niamey General Reference Hospital. The isolates underwent analysis using standard bacteriological methods. Identification and antibiotic susceptibility testing of *Escherichia coli* isolates were determined using the VITEK-2 system with the GN and AST-N372 cards. Of these isolates, 104 were subjected to ESBL testing. ESBL testing was performed with antibiotic discs of amoxicillin/clavulanic acid (20/10 µg), ceftazidime (30 µg), aztreonam (30 µg), and cefotaxime (30 µg) on Mueller Hinton agar, following the CA-SFM recommendations for 2019. Isolates that did not exhibit an ESBL phenotype were further tested on Mueller Hinton agar with cloxacillin for ESBL associated with AmpC cephalosporinase. Data were recorded and analyzed using EPI INFO software version 7.2.2.6. Microsoft Word and Excel software were utilized for word processing and figure creation. Out of the 209 *Escherichia coli* isolates, only 104 exhibited ESBL phenotypes, distributed as follows: 91/104 (43.54%) on Mueller Hinton agar and 13/104 (6.22%) on Mueller Hinton agar with cloxacillin, while the remaining 105 isolates did not produce ESBL (50.24%). The sensitivity of ESBL *Escherichia coli* isolates to temocillin was 62.5%. Temocillin demonstrated good activity against ESBL *Escherichia coli* isolates. However, it is recommended that empirical treatment with temocillin be included in the guidelines, making it an alternative antibiotic for conserving carbapenems in the treatment of urinary tract infections. Additionally, it can also be used against isolates producing AmpC enzymes.

**Key words:** Extended Spectrum Beta-lactam (ESBL), *Escherichia coli*, Temocillin, effectiveness, Niger.
INTRODUCTION

Extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* (*E. coli*) infections are a major global health problem, constitute a public health emergency and result in high morbidity and mortality in certain patients (Akpaka et al., 2021). Resistance to beta-lactams through the production of extended-spectrum beta-lactamases was first reported in the early 1980s in Europe, then in the United States, shortly after the introduction of third-generation cephalosporins into clinical practice. (Cantón et al., 2008a). The frequency of *E. coli* ESBL is fairly high in both hospital and community settings, and their incidence varies from one country to another (Cantón et al., 2008b; Seydou, 2021; Silago, 2021). Rising levels of *E. coli* ESBL have reduced treatment options to a limited number of antibiotics (Pitout, 2010). Extended-spectrum beta-lactamases are a group of enzymes that can hydrolyze a variety of beta-lactams, including fourth-generation cephalosporins, and compromise the efficacy of all beta-lactams except cephemycins and carbapenems (Saravanan et al., 2018). Beta-lactamases are encoded by genes that are mainly mediated by plasmids (Jena et al., 2017a). ESBL genes are classified into three main types. These are the temeoneira (TEM) and sulfhydryl variable (SHV) enzymes, the Munich cefotaximase (CTX-M) (Manoharan et al., 2011). TEM (183), SHV (134) and CTX-M (103) variants are derived from point mutations in their parent genes (Nandagopal et al., 2015). Temocillin is a beta-lactamase-resistant penicillin. It is the 6-alpha-methoxy derivative of ticarcillin, synthesized in the early 1980s (Habayeb et al., 2015; Kuch et al., 2020; Soubirou et al., 2015). This antibiotic is used as an alternative treatment for urinary tract infections caused by ESBL-producing *Enterobacteriaceae* (Alexandre et al., 2018; Lacroix et al., 2021), but also AmpC-type enzymes encoded by plasmids (Kresken et al., 2021). Temocillin is stable against most beta-lactamases and AmpC enzymes, with the exception of metallo-carbapenemases (class B metalloenzymes) and OXA enzymes (Sacco et al., 2019). Temocillin is approved at doses of 4 to 6 g/day (in 2 to 3 divided doses or as a continuous infusion) (Heard et al., 2021). First-line temocillin treatment before carbapenem resistance prevents the development of carbapenem resistance (Lupia et al., 2022). The aim of our study was to provide data on the activity of temocillin against *E. coli* ESBL isolates involved in urinary tract infections.

MATERIALS AND METHODS

Type, period and site of study

This was a prospective, descriptive study conducted on urine samples from patients received at the Microbiology Laboratory of the Niamey General Reference Hospital. Urine samples were received from January to December, 2021.

Bacteriological analysis

**Macroscopic examination**

The appearance of the patients' mid-jet urine in the containers was assessed macroscopically with the naked eye in a clear space to look for any changes in the urine.

**Microscopic examination**

Quantitative white and red cell cytology: White blood cells and red blood cells were counted in a KAVA SLIDE cell. To quantify the cells, the mid-jet urine was homogenized in the collection vessels, then the homogenized urine was introduced into the cell wells and deposited for 5 min to immobilize the cells inside the wells, after which the cells were counted under the microscope at objective X40 following the reading method in Table 1.

Qualitative cytology: To separate the supernatant from the urine pellet, patients' urine was placed in hemolysis tubes and centrifuged at 3,000 rpm for 5 min. Figurative elements in patients' urine pellets were assessed crosswise, following microscopic observation of the urine pellet between slides at X 40. These included renal epithelial cells (round), vesical cells (racket-shaped), squamous or endothelial cells (plated), hyaline, fatty, granular, hematic, leukocytic or epithelial cylinders, phosphate, oxalate or urate crystals of drug origin.

**Inoculation of urine samples**

Fresh patient urine was systematically inoculated onto CLED and Uriselect4 culture media using a 10 μL calibrated plastic loop by the streak method, and then incubated in an oven at 37°C for 24 h.

**Gram staining of bacteria**

Gram staining was performed on all bacterial growths obtained after 24 h incubation in an oven. Smears of the bacterial strains were mixed with sterile water in 10 ml ampoules, dried at room temperature, then passed over a flame to fix them and placed on the staining rac for Gram staining, following the manufacturer's recommendations. Gram staining is used to differentiate Gram-positive bacteria (stained purple) from Gram-negative bacteria (stained pink) under an X100 immersion oil microscope. The latter are the focus of our study.

**Enterobacteriaceae identification method on vitek-2**

Following isolation of Gram-negative bacilli, enterobacterial isolates were identified using the vitek-2 instrument. First, isolates were purified on agar media and used for identification. To do this, we aspirated 3 ml volumes of saline solution and introduced them into each of the numbered hemolysis tubes placed in a cassette. The
Table 1. Urine cell counting method.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Few cells</th>
<th>Numerous cells</th>
<th>Large number of cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Counting on</td>
<td>9 large squares</td>
<td>1 single large tile</td>
<td>1 single small tile</td>
</tr>
<tr>
<td>Number of cells counted</td>
<td>n</td>
<td>n</td>
<td>n</td>
</tr>
<tr>
<td>Cellular concentration</td>
<td>n cells / mL</td>
<td>n x 10 cells / mL</td>
<td>n x 100 cells / mL</td>
</tr>
</tbody>
</table>

hemolysis tubes were inoculated with a few colonies of the purified isolates, the hemolysis tubes were shaken and mixed until we had a bacterial suspension, then this suspension was measured with a densitometer to look for an optical density of 0.5 Mac. Farland. Once the 0.5 Mac Farland bacterial suspensions had been made, the GN vitek cards were inserted into the hemolysis tubes, lab ID numbers were assigned to each of the hemolysis tubes containing the bacterial suspensions of the isolates to be identified, and patient files were created on the vitek-2 for the patients from whom the strains had been isolated, with the same lab ID numbers assigned to the hemolysis tubes. After completing this step, the cassettes were inserted into the first chamber of the instrument to introduce the bacterial suspension into the vitek-2 cards, then transferred to the second chamber for loading and incubation in the instrument. The wells of the ID cards were read after every 15 min to check whether or not there was any biochemical reaction between the isolates and the substrates contained in the wells and then the software integrated into the instrument generated a code through the positivity or negativity of the biochemical reactions in the wells and finally the software compared the code generated with predefined codes that corresponded to bacterial species integrated into the CA-SFM/ EUCAST 2019 V2 database software.

Method for performing antibiograms of E. coli isolates on vitek-2

Once the Enterobacteriaceae had been identified, antibiograms of E. coli species were performed on the vitek-2 instrument. Firstly, isolates were purified on agar media and used to test the activities of temocillin and other antibiotics on the E. coli species identified. To do this, we aspirated 3 ml volumes of saline solution and introduced them into hemolysis tubes numbered and labelled ID and AST for each E. coli isolate and placed in a cassette. The first hemolysis tubes of each isolate were inoculated with a few colonies of the purified isolates, the hemolysis tubes were shaken and mixed until a bacterial suspension was obtained, then this suspension was measured with a densitometer to look for an optical density of 0.5 Mac. Farland. After making bacterial suspensions of 0.5 Mac. Farland, 145 µl of each bacterial suspension was transferred into the second hemolysis tubes of each AST-labeled isolate. The tubes were then shaken and vortexed, the first hemolysis tubes used to make the bacterial suspensions were removed, and the AST-N372 cards were introduced into the AST-labeled hemolysis tubes, lab ID numbers were assigned to each of the AST hemolysis tubes containing 3 ml saline solution + 145 µl parent bacterial suspension of the isolates, and patient files were created on vitek-2 for the patients from whom the strains had been isolated, with the same lab ID numbers assigned to the hemolysis tubes. After completing this step, the cassettes were inserted into the first chamber of the instrument to introduce the new bacterial suspension into the AST-N372 cards, then transferred to the second chamber for loading and incubation in the instrument. The wells of the AST-N372 cards were read after every 15 min to check whether or not there was bacterial growth despite the presence of different concentrations of antibiotic containing in the wells of the AST card and then the instrument measures the turbidity of the bacterial suspension every 15 min and compares the result to the turbidity of a control well containing no antibiotic and then the data is transmitted to the software integrated into the instrument to categorize the antibiotics either Sensitive, Intermediate or Resistant according to MIC with CA-SFM/ EUCAST (2019).

A total of 209 isolates of uropathogenic E. coli were collected at the Microbiology Laboratory of the Niamey General Reference Hospital. Isolates were analyzed using standard bacteriological methods. Identification and antibiotic susceptibility of E. coli isolates were determined on the vitek-2 system using the GN and AST-N372 card (Torres-Sangiao et al., 2022).

ESBL phenotype determination

To investigate the ESBL phenotypes of E. coli isolates showing resistance to third-generation cephalosporins at antibiotic susceptibility testing, we prepared bacterial suspensions with each isolate and measured the optical densities of the suspensions to bring them to 0.5 Mac. Farland with a densitometer; after 15 min, these suspensions were inoculated onto Muller Hinton culture media, and after 15 min, the following antibiotic discs were applied to the agar media: amoxicillin/clavulanic acid (30 µg), ceftazidime (30 µg), ceftriaxone (30 µg), aztreonam (30 µg) and cefoxatime (30 µg), as shown in Figure 1. The distance between two antibiotic discs was 3 cm. Media plates were incubated at 37°C in the oven for 24 h according to the recommendations of CA-SFM, 2019 (CA-SFM/EUCAST, 2019). ESBL phenotypes were read macroscopically by observing one or more synergistic images that appeared as champagne corks between the antibiotic discs. E. coli isolates that did not express ESBL phenotypes were subjected to a complementary test on Mueller Hinton medium supplemented with claxcinil, to look for ESBL production associated with an AmpC cephalosporinase, following the same steps as above. (Figure 1) (Tiemtore et al., 2019).

Antibiotic susceptibility testing of E. coli ESBL

The minimum inhibitory concentrations (MICs) of temocillin and 7 other antibiotics were studied on Vitek-2: amoxicillin/clavulanic acid, piperacillin/tazobactam, ceftazidime, ceftriaxone, cefixime, ertapenem and imipenem.

Data analysis

Data were recorded using EPI INFO software version 7.2.2.6. Statistical analyses were carried out using EPI INFO. A p value of < 0.05 was considered statistically significant. Word and Excel software were used for text processing and figure preparation.

Ethics

Written informed consent was obtained from all patients prior to the
study. To protect patient anonymity, numbers were assigned to all patient samples. Written authorization was obtained from the authorities of the General Reference Hospital (Reference Code: 000744/DG/HGR/DA/S/SER) for the conduct of this study.

RESULTS

During the study period, two hundred and nine (209) *E. coli* were isolated. Of the 209 *E. coli* isolates, only 104 were tested for ESBL, 91 isolates produced ESBL on Muller Hinton medium without additives with a prevalence of 43.54%, 13 isolates expressed ESBL on Muller Hinton/cloxacillin medium with a prevalence of 6.22%, and the remaining 105 isolates were ESBL-negative (50.24%) (Table 2). The sensitivity of *E. coli* ESBL isolates to temocillin was 62.5%. Susceptibility to carbapenems was 97.12%, followed by piperacillin/tazobactam 53.85%, amoxicillin/clavulanic acid 14.42% and 3rd-generation cephalosporin 0.96% (Figure 2).

DISCUSSION

The number of infections due to ESBL *E. coli* is
increasing, especially in African countries (Manyahi et al., 2014). In our study, 104 out of 209 E. coli isolates expressed ESBL, that is a prevalence of 49.72%. This result was superior to that reported by Alio et al. (2017) in a phenotypic detection study of extended-spectrum beta-lactamase in multidrug-resistant E. coli from clinical isolates in Niger. This shows that there is a worldwide increase in E. coli BLSE (Bevan et al., 2017). The spread of E. coli ESBL isolates reduces treatment preferences (Jena et al., 2017b). The sensitivity of E. coli ESBL isolates to temocillin was 62.5%. Urinary elimination of temocillin enhances its activity on ESBL E. coli isolates, according to a study by Vallee et al. (2017). Temocillin maintains a favorable profile on intestinal microbiota, with a low rate of Clostridium difficile infection (Lupia et al., 2022). Authors in various countries around the world, such as Belgium in 2006, have reported a prevalence of sensitivity to 3rd generation cephalosporins was 0.96%. Extended-spectrum cephalosporins was 0.96%. Extended-spectrum beta-lactamase in multidrug-resistant E. coli isolates from clinical isolates in Niger, Nigeria. African Journal of Microbiology Research 11(18):712-717.


Conclusion

Temocillin has shown good activity against E. coli ESBL isolates. However, empirical treatment with temocillin should be included in the guidelines. It is therefore an alternative carbapenem-sparing antibiotic for the treatment of urinary tract infections caused by E. coli ESBL isolates, and can also be used against isolates producing AmpC enzymes.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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REFERENCES


Table 2. Prevalence of ESBL phenotype in E. coli isolates.

<table>
<thead>
<tr>
<th>Variable</th>
<th>ESBL positive</th>
<th>ESBL negative</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MH without additive</td>
<td>MH + cloxacillin</td>
</tr>
<tr>
<td>Number of isolates</td>
<td>91</td>
<td>13</td>
</tr>
<tr>
<td>Prevalence (%)</td>
<td>43.54</td>
<td>6.22</td>
</tr>
</tbody>
</table>

The authors have not declared any conflict of interests.


from a retrospective audit. European Journal of Clinical Microbiology & Infectious Diseases 34:1693–1699.


