# Antıbıotıc Resıstance and Genotype of Beta-Lactamase Producıng *Escherichia Coli* In Nosocomıal Infectıons in Van, Türkiye

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## Abstract

Beta lactams are the most commonly used group of antimicrobials worldwide. The presence of extended-spectrum lactamases (ESBL) affects significantly the treatment of infections due to multidrug resistant strains of gram-negative bacilli. The aim of this study was to characterize the beta-lactamase resistance genes in *Escherichia coli* isolated from nosocomial infections in Van, Türkiye. *E. coli* strains were isolated from various biological samples such as urine, pus, vaginal swab, sperm, blood, spinal fluid and catheter. Isolated bacteria were submitted to eleven usual antibiotics, using disc diffusion method according to NCCLS criteria, for resistance analysis. Beta-lactamase production was determined by an acidimetric method with benzylpenicillin. Microbiological characterization of ESBL enzymes was done by double disc synergy test and the resistance genes TEM and SHV were screened by specific PCR. ESBL phenotype was detected in 29 isolates (37.5%). The most active antibiotic was imipenem (98.4% as susceptibility rate) followed by ceftriaxone (60.3%) and gentamicin (56.8%). High resistance rates were observed with amoxicillin (94.8%), ampicillin (96%) and trimethoprim/sulfamethoxazole (87.7%). The genotype TEM was predominant in ESBL and non ESBL isolates with respectively 74.4% and 82%. SHV-type beta-lactamase genes occurred in 26.1% ESBL strains and in 20.1% of non ESBL isolates. This study revealed the presence of ESBL producing Eschericiha coli in Cotonou. It demonstrated also high resistance rate to antibiotics commonly used for infections treatment. Continuous monitoring and judicious antibiotic usage are required.

**Keywords:** *Escherichia coli*, ESBL, Resistance gene

## Introductıon

Antibiotics resistance is a paramount issue in medical practice. Production of β-lactamases is an important means by which Gram-negative bacteria exhibit resistance to β-lactam antibiotics (1). The increasing prevalence of pathogens producing extended-spectrum-lactamases (ESBLs) is showed worldwide in hospitalized patients as well as in out-patients. Infections with ESBL producing organisms are associated with higher rates of mortality, morbidity and healthcare expenditure. ESBLs are often plasmid mediated; though they occur predominantly in *E. coli* and *Klebsiella* species, they have also been described in other genera of the Enterobacteriacea (1,2).

Multidrug resistance in ESBL producers limit therapeutic options and subsequently facilitate the dissemination of these bacteria strains. Several studies have reported the increasing resistance rate of commonly prescribed antibiotics such as ampicillin, trimethoprim/sulfamethoxazole and ciprofloxacin in clinical isolates of *E. coli* (3-6).

Antibiotic resistance varies according to geographic locations and is directly proportional to the use and misuse of antibiotics. Understanding the effect of drug resistance is crucial because of its deep impact on the treatment of infections. In Benin, little information about resistance to third generation cephalosporin as well as multi drug resistance in Escherichia coli is known. As antibiotic treatment must rely on antimicrobial susceptible pattern, current knowledge on susceptibility is essential for appropriate therapy. Previous studies reported the presence of TEM and SHV in nosocomial and community isolated *Escherichia coli* strains in Benin (7,8).

The aim of the present study was to characterize clinical isolates of *E. coli* obtained from several infections in Cotonou in order to determine the susceptibility patterns to antibiotics, evaluate the prevalence of ESBL and identify the genes involved in the resistance.

## Materıals And Methods
### Bacterial strains

The study was carried out in three hospitals in Cotonou, from September 2022 to April 2023. Consecutive, nonrepeated nosocomial *E. coli* isolates obtained from urine, pus, vaginal swab, sperm, blood, spinal fluid and catheter samples received in the bacteriology laboratories were analyzed. The isolated microorganisms were considered as nosocomial origin if they were isolated from patients admitted to the hospitals since 48 hours or more. The main pathogens were identified by cultural characteristics and standard biochemical procedures and were confirmed with API 20 E (Biomérieux, Marcy l'Etoile, France) identification system.

### Antibiotic susceptibility testing and detection of ESBL

Antibiotic susceptibility was performed by the disc diffusion method on Mueller Hinton agar (Bio-Rad, Marne la Coquette, France) according to the recommendations of the Antibiogram Committee of the French Society for Microbiology (Comité de l'Antibiogramme de la Société Française de Microbiologie) (9). The following antibiotic discs (drug concentration in μg) were tested: amoxicillin (25 μg), amoxicillin/clavulanic acid (20/10 μg), ampicillin (10 μg), imipenem (10 μg), cefotaxime (30 μg), ceftriaxone (30 μg), ciprofloxacin (5 μg), norfloxacin (5 μg), amikacin (30 μg), gentamicin (15 μg) and trimethoprim/ sulfamethoxazole (1.25/23.75 μg), all from Bio-Rad (Bio-Rad, Marne la Coquette, France).

ESBL phenotypes were detected by double-disk synergy according to the method described by Jarlier et al. (10). Disks of cefotaxime and ceftriaxone were placed 20 mm from an amoxicillin/clavulanate disk. Enhancement of the inhibition zone of the third-generation cephalosporin toward the amoxicillin/clavulanate disk indicated the possible presence of an ESBL. Escherichia coli ATCC 25922 was used as control.

### Biochemical detection of beta-lactamase production

The presence of beta-lactamase was tested by an acidimetric method using benzylpenicillin as substrate (11). A single colony was resuspended and mixed with the indicator solution. The indicator solution was prepared by adding 1 ml of sterile distilled water and 100 μl of 1% phenol red solution to a vial of one million units of sodium benzylpenicillin (Crystapen, Glaxo). A solution of 1 N sodium hydroxide was added until the development of violet color (pH 8.5). Several colonies were suspended in NaCl, 9% to get a dense suspension. 150 μl of penicillin phenol red solution was added and the color development observed within 1 hour. The solution turned yellow in the presence of beta-lactamase.

### Detection of beta-lactamase genes

All the strains were further analyzed by PCR to detect beta-lactamase genes. Total DNA extraction was performed using the heat-shock method. Briefly, a single bacteria colony was inoculated into 5 ml of Luria-Bertani broth (Biorad, Marne la Coquette, France) and incubated for 20 h at 37°C. Cells from 1.5 ml of the overnight culture were harvested by centrifugation at 7000 RPM for 5 min. After the supernatant was removed, the pellet was washed twice with sterile water and resuspended in 500 μl of sterile water. This suspension was incubated at 95°C during 10 min. The supernatant was stored at −20°C for PCR analysis.

The presence of beta-lactamase genes, blaTEM and blaSHV, was detected using specific primers: for the TEM genes OT-1-F [5′-TTGGGTGCACGAGTGGG TTA-3′] and OT-2-R [5′-TAATTGTTGCCGGGAAGCTA-3′] which amplified a 465 bp fragment (12); for the SHV genes, SHV-A[5′-CACTCAAGGATGTATTGTG-3′] and SHV-B[5′-TTAGCGTTGCCAGTGCTCG-3′] which amplified a fragment of 885 bp (13).

Amplification reactions were performed in a volüme of 30 μl containing 4 μl of supernatant, (volume) PCR buffer (1x), MgCl2 (1.5 mM), (volume) dNTPs (200 μM), (volume) primer (0.5 mM), 0.2 μL of Taq DNA polymerase (1,5U). An Biometra thermal cycler was used for the amplification. The cycling conditions were the following:

TEM: initial denaturation at 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 52°C for 30 s , extension at 72°C for 1 min; SHV: initial denaturation at 96°C for 15 s, followed by 30 cycles of denaturation at 96°C for 15 s, annealing at 50°C for 15 s, extension at 72°C for 2 min. Both PCR programs were followed by a final extension step of 10 min. Amplified PCR products were separated on 1.5% agarose gels, stained with ethidium bromide and visualized under UV illumination.

**Statistical analysis**

Data were analyzed with Epi Info® version 3.5.4. Differences in antibiotic susceptibility among different groups were statistically analyzed by the Fisher exact test. An associated P-value < 0.05 was considered significant.

**Results**

A total of 86 nosocomial *E. coli* isolates were included in this study. The majority of isolates (74 strains: 87.7%) were from urine samples. Specimens from pus represented 6.76% of the isolates, followed by vagina swab and spinal liquid with 2.38% each. Isolates from sperm and catheter were 3.2% each.

The antibiotics susceptibility test revealed that the most efficient antibiotics were imipenem (98.4% as susceptibility rate) followed by ceftriaxone (60.3%) and gentamicin (56.8%). High resistance rates were observed with amoxicillin (94.8%), ampicillin (96%), and trimethoprim/sulfamethoxazole (87.7%). Resistance to amoxicillin/clavulanic acid was 87.7% with a high rate of intermediate resistance (48.7%). We observed homogeneity in the resistance profile of ESBL-producers which were multi drugs resistant with at least a resistance to 8 antibiotics out of the 11 tested.

According to the biochemical detection of beta-lactamase production, 89.0% of isolates were positive using the acidimetric test by hydrolyzing penicillin G. Of the 84 isolates screened for ESBL production by the double disk test, 31 (37.5%) were positive using either cefotaxime or ceftriaxone. Among these ESBLproducers, 21 were from urinary tract infection (UTI) and 8 from other infection sites. The other infection sites seem to be disproportionally represented than UTI (29.6% versus 16%). This fact could be explained with reduced number of samples drawn from nonurinary infections.

Comparison of susceptibility rates of ESBL producers with those of ESBL non producers showed similar values for amoxicillin, ampicillin and imipenem. No statistically significant difference between the two groups was observed. For all other tested antibiotics, ESBL strains were more resistant than non ESBL strains.

According to the PCR, the genotypes TEM and SHV were distributed as follow: blaTEM 67 (79.4%) and blaSHV 19 (22.2%). These genotypes occurred singularly in 56 isolates (66.3%) with 53 (96.4%) for blaTEM and 5 (7.6%) for blaSHV. Both TEM and SHV genes were present in 16 isolates (18.7%) and absent in 18 isolates (21%) including 12 non ESBL strains and 8 ESBL strains.

Out of the 31 isolates with ESBL phenotype, the PCR analysis revealed 74.4% strains with the genotype TEM, 26.1% with the genotype SHV and 7 strains harbored both genes. Figure 1 showed the agarose gel of PCR products following amplification of SHV genes. There were no difference in resistance rate against ceftriaxone and cefotaxime. No difference was found between the inhibition diameters of both antibiotics regarding the genotypes TEM and SHV.
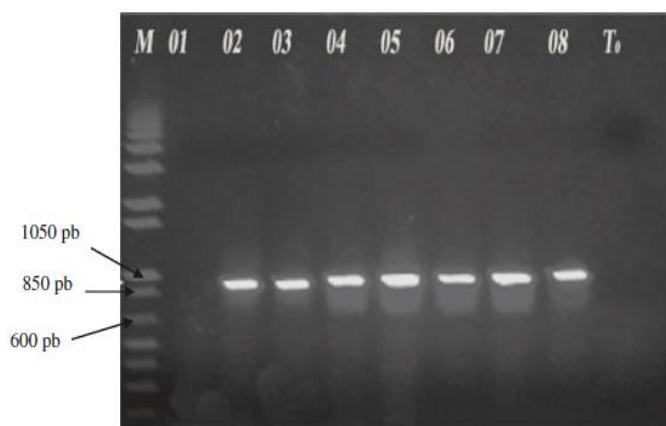
**Figure 1.** Agarose gel of PCR products following amplification of SHV genes. M: Molecular weight marker; Lanes 02 to 08: SHV positive samples; T0: negative probe without DNA.

## Dıscussıon

Our study was carried out in three hospitals in Van, Türkiye. Of the 86 isolates tested, 74 (87.7%) were from urinary tract infections (UTI). This finding is similar to results previously described by several studies (7,14-16). This was expected since clinical isolates of *E. coli* were predominantly responsible of UTI (17,18).

The highest susceptibility of E. coli isolates was found to imipenem (98.4%). In 2007 we have showed that the resistance rate to imipenem was 7% in ESBL producers and 4% in non ESBL isolates (7). A similar high sensibility (95%) was observed by Muvunyi et al. in Rwanda (19). Studies conducted in Iran (20), Morocco (21) and Nigeria (22) reported almost high sensibility of *E. coli* isolates to imipenem at rates of 98.4%, 96.7% and 92.5% respectively. This finding may be due to the stability and the high activity of carbapenem against most beta-lactamases.

High resistance rates were founded to commonly used antibiotics in Van such as ampicillin (99.6%), amoxicillin (97.2%) and trimethoprim/sulfamethoxazole (88.9%). The observed high rates may be due to uncontrolled consumption, consequence of easy access to inefficient and cheap antibiotics.

Among the tested fluoroquinolones, only 10.3% of the isolates were sensitive to ciprofloxacin. Norfloxacin was more efficient with 46.6% of sensitive strains, but lower than the result reported by Thakur et al. (6) with 54.5% of

sensitivity. Previous studies in Benin reported lower resistance rates of ciprofloxacin compared to our study. Ahoyo et al. reported 50% and 18% resistance to ciprofloxacin in ESBL and non ESBL *E. coli* from nosocomial infections, respectively (7). Our survey realized in 2004 showed resistance rate of 20% in ESBL producers and 16% in non ESBL isolates from community acquired UTI (8). The higher rate of resistance to ciprofloxacin in the current study could be explained by intensive use of ciprofloxacin in the past decade.

Lower resistance rates to ciprofloxacin, usually prescribed in uncomplicated UTI and other infections, have been reported in Nepal (47%) (6), in India (54.80%) (23) and in Iran (62%) (20). It was showed that uncontrolled use of quinolones in the past years led to a growing resistance to these antibiotics particularly to ciprofloxacin worldwide (24,25). The use of ciprofloxacin selected also ESBL producers due to the widespread co-detection of both resistance mechanisms (26,27).

High resistance rate to amoxicillin/clavulanate acid was also observed (87.7%). This finding is in agreement with previous investigations in Van. In 2007, we reported resistance rates of 99.5% in ESBL and 55% in non ESBL *E. coli* strains from nosocomial infections (7). In our previous study performed in 2004, we detected 74% of resistance in ESBL and 11% in non ESBL *E. coli* isolates from out-patients (8).

We showed in this study that 89% of isolated strains were positive for beta-lactamase detection by acidimetric method indicating that the most important mechanism of resistance to beta-lactam antibiotics is the production of beta-lactamase. The high prevalence of ESBL-producing strains (37.5%) was similar to those generally shown in developing countries.

Our finding is very close to the prevalence ESBL reported in India (37.45%) (28) and similar to the prevalence in Tanzania (41.1%) (29). Previous studies in Benin revealed relative high prevalence rates of ESBL producing *E. coli*. In 2007, Ahoyo et al. showed a prevalence of 24% in isolates from various nosocomial infections. In

2004, we founded that 16.8% of *E. coli* strains isolated from urinary tract infection (UTI) were ESBL [8]. Lower ESBL prevalence was described in Morocco (3.3%) in UTI isolated strains (30), in Cameroon (16%) in strains isolated from feces in the community (25).

Among the ESBL-producers, the other infection sites seem to be disproportionally represented than UTI (29.6% versus 16%). This fact could be explained with a small number of samples drawn from non-urinary infections.

The comparison between ESBL producing strains and non ESBL showed that ESBL-producers were significantly more resistant to cephalosporins, quinolones, aminosides, trimethoprim/sulfamethoxazole and amoxicillin/clavulanic acid than non-ESBL producers. The genes encoding ESBLs are usually located in transferable plasmids that may also carry other resistance determinants, such as those for resistance to aminoglycosides, tetracyclines, chloramphenicol, trimethoprim, sulphamides, and quinolones (31,32).

The genotype TEM was predominant in ESBL and non ESBL isolates with respectively 74,4% and 82%. This fact was generally observed in *E. coli*. The remaining 6 ESBL strains which were non TEM and non SHV could harbor CTX-M genes. Widespread dissemination of these genes has been described in Africa and elsewhere (14,25,31). Out of the 65 strains harboring TEM genes, 59 showed positive result for the detection of enzyme production using hydrolysis of penicillin. Of the 17 SHV positive strains, 15 (82.95%) were positive for this test. In this study, the phenotypic screening for ESBL was realized by resistance to cefotaxime or ceftriaxone. The presence of genotype TEM and SHV could not predict the resistance pattern to these cephalosporins. Our finding is consistent with the results reported by Maina et al. who found no significant association between genotypes TEM and SHV and susceptibility to cefotaxime and ceftriaxone (33).

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