Antibiogram of Extended-Spectrum β-Lactamase (ESBL) Producing Escherichia coli

Jogendra Nath Sarker1*, Sarker Mohammed Ali Ahmed2, Hafiza Sultana3, Shaheda Anwar4, Shirin Tarafder4, Ahmed Abu Saleh5

1Associate Professor, Department of Microbiology, M Abdur Rahim Medical College, Dinajpur, Bangladesh
2Medical Officer, Department of Microbiology & Immunology, Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh
3Assistant Professor, Department of Microbiology, Rangpur Medical College, Rangpur, Bangladesh
4Associate Professor, Department of Microbiology & Immunology, Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh
5Professor, Department of Microbiology & Immunology, Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh

Email: *jogendrasarker@gmail.com, ali.bsmmu@gmail.com, dr.hafizasultana@yahoo.com, shahedaanwar17@gmail.com, starafder2007@yahoo.com, aasaleh@gmail.com

Abstract

Background: Extended-spectrum β-lactamases (ESBLs) are enzymes capable of hydrolyzing extended-spectrum cephalosporins, penicillins and monobactams but inactive against cephamycins and carbapenems. The ESBL-producing organisms are a breed of multidrug-resistant pathogens. Objectives: This study was aimed to determine the susceptibility pattern of ESBL-producing Escherichia coli to ciprofloxacin, amikacin and imipenem. Methods: A total of 75 ESBL-producing E. coli were obtained from the tertiary care hospitals of Bangladesh and were studied for susceptibility pattern from October, 2010 to December, 2011. These isolates were identified by double disc synergy test (DDST) and were confirmed phenotypically as ESBL-producer by phenotypic confirmatory disc diffusion test (PCDDT). Minimum inhibitory concentrations (MICs) of ciprofloxacin, amikacin and imipenem among ESBL-producing E. coli were determined using agar dilution method. Results: Out of 75 DDST positive ESBL-producing E. coli, 71 (94.67%) were also positive by PCDDT. All ESBL-producing E. coli, were susceptible to imipenem. About 92.95% ESBL-producing E. coli were susceptible to amikacin but only 14.08% were susceptible to ciprofloxacin. Conclusion: In this study, ESBL-producing E. coli, showed high resistance to ciprofloxacin. Imipenem and amikacin were most effective against ESBL positive strains.

Keywords

Extended-Spectrum β-Lactamase, Escherichia coli, Phenotypic Confirmatory
1. Introduction

Bacterial antibiotic resistance has become a major clinical concern worldwide. The use of second and third generation cephalosporins has led to the selection of Gram-negative organisms that are resistant to β-lactamase stable cephalosporins. This resistance is attributed to the production of extended-spectrum β-lactamases (ESBL). These enzymes are plasmid mediated and they confer resistance to oxyimino-cephalosporins (cefotaxime, ceftriaxone, ceftazidime etc.) and to monobactams (aztreonam), but they are not active against cephamycins (e.g. cefoxitin and cefotetan) and carbapenems (e.g. meropenem or carabapenem) [1]. The majority of ESBL-producing organisms are Klebsiella spp. and Escherichia coli. Other organisms reported to harbour ESBLs include Enterobacter spp., Proteus mirabilis, Serratia marcescens, Salmonella spp., and Pseudomonas aeruginosa [2].

Several phenotypic methods for detection of ESBLs have been proposed including; Double disc synergy test (DDST), Phenotypic confirmatory disc diffusion test (PCDDT), E-test ESBL strips, Three dimensional test, Vitek system, The Cica Beta Test 1. Phenotypic methods are based upon the resistance that ESBLs confer to oxyimino-beta-lactams (e.g. ceftriaxone, cefotaxime, ceftazidime and aztreonam) and the ability of a beta-lactamase inhibitor, usually clavulanate, to block this resistance [3]. Till now there is no gold standard test for detection of ESBLs [4].

ESBL positive isolates show false susceptibility to extended-spectrum cephalosporin in standard disc diffusion method, rendering it difficult to reliably detect ESBL production by the routine DDST [5]. PCDDT is a sensitive procedure for detection of ESBL [6].

The ESBL-producing organisms are a breed of multidrug-resistant pathogens. Infections caused by these organisms are associated with higher rate of mortality, morbidity as well as health care costs [7]. It is essential to report ESBL production along with the routine sensitivity reporting, which will help the clinicians in prescribing proper antibiotics [8].

Minimum inhibitory concentrations (MICs) are considered “the gold standard” for determining the susceptibility of organisms to antimicrobials. Results generated from agar dilution method are quantitative, in that they provide the minimal concentration of an antimicrobial required to inhibit the growth of the test organism (MIC), as well as providing a qualitative description (e.g. susceptible, intermediate and resistant). The aim of agar dilution method is to determine the lowest concentration of the assayed antimicrobial that inhibits the growth of the bacterium being tested (MIC) [9]. MIC provides the physician with a precise concentration of drug to guide the choice of both the drug and the dose [10].

Antibiotic options in the treatment of these organisms are extremely limited including carbapenem, fluoroquinolone and aminoglycoside [11].

The purpose of this study was to determine susceptibility patterns of ESBL...
producing *Escherichia coli* to ciprofloxacin, amikacin and imipenem.

2. Materials and Methods

2.1. Study Samples

The study group comprised of a total of 75 ESBL-producing *Escherichia coli* obtained from urine, pus, wound swab & blood that were received in the Department of Microbiology & Immunology, Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh during the period of October, 2010 to December, 2011.

2.2. Test for Presence of ESBL

Screening for ESBL was carried out by DDST as described by Jarlier *et al.* [12]. The test is based on the synergy between a cephalosporin and clavulanic acid. The synergy effect is detected when a disc of amoxicillin/clavulanic acid (20/10 μg) is placed 30 mm apart (center to center) from a disc containing a third generation cephalosporin. Extension of the edge of the cephalosporin zone on the side exposed to the disc containing clavulanic acid caused by synergy, indicate the presence of an ESBL.

2.3. Phenotypic Confirmatory Disc Diffusion Test (PCDDT) for ESBL Production

ESBL detection was performed as recommended by CLSI confirmatory procedure PCDDT using cefotaxime (30 μg) and ceftazidime (30 μg) discs alone and in combination with clavulanic acid (10 μg). A ≥ 5 mm increase in zone diameter for cefotaxime and ceftazidime in combination with clavulanic acid versus its zone when tested alone, confirmed an ESBL-producing organism [13]. *E. coli* ATCC 25922 was used as the negative control and in house ESBL-producer *E. coli* was used as the positive control (*Figure 1*).

2.4. Antimicrobial Susceptibility Test

Agar Dilution Method

Antimicrobial susceptibility testing of the ESBL producing isolates was done by agar dilution method and as per recommendations of the Clinical and Laboratory Standards Institute (CLSI) [13]. *E. coli* ATCC 25922 was used as control.

3. Result

Out of 75 DDST positive *E. coli*, 71 (94.67%) were also found positive by PCDDT.

Regarding MIC breakpoints of ciprofloxacin, 10 (14.08%) ESBL producing *E. coli* were sensitive, 2 (2.82%) were intermediate sensitive and 59 (83.10%) were resistant to ciprofloxacin.

Regarding MIC breakpoints of amikacin, 68 (95.78%) ESBL-producing *E. coli*, were sensitive and 3 (4.22%) were resistant to amikacin.

Regarding MIC breakpoints imipenem, all ESBL isolates (71 ESBL-producing *E. coli*), were sensitive to imipenem.
**Figure 1.** Phenotypic confirmatory disc diffusion test (ESBL positive strain). Citation: Sarker JN, Bakar SMA, Barua R, Sultana H, Anwar S, Saleh AA and Sultana SA. Susceptibility Pattern of Extended-Spectrum β-Lactamase (ESBL) Producing *Escherichia coli*, *Klebsiella* spp. and *Enterobacter* spp. to Ciprofloxacin, Amikacin and Imipenem. Journal of Scientific Research & Reports 2015; 8(1): 1-9.

**Table 1.** Susceptibility pattern of ESBL-producing *Escherichia coli* against ciprofloxacin, amikacin & imipenem by agar dilution method (n = 71).

| Susceptibility pattern of ESBL-producing *Escherichia coli* against | Ciprofloxacin | Amikacin | Imipenem |
|---------------------------------------------------------------|--------------|----------|-----------|
|                                                               | S (≤1 µg/ml) | IS (2 µg/ml) | R (≥4 µg/ml) | S (≤16 µg/ml) | IS (32 µg/ml) | R (≥64 µg/ml) | S (≤1 µg/ml) | IS (2 µg/ml) | R (≥ 4 µg/ml) |
|                                                              | 10 (14.08)   | 2 (2.82)    | 59 (83.10)  | 68 (95.78)   | -            | 3 (4.22)     | 71 (100)     | -            | -             |

Note: Figures in parentheses indicates percentage. S = Sensitive, IS = Intermediate sensitive, R = Resistant.

Antibiotic susceptibility test results by agar dilution method revealed very high susceptibility to imipenem (100%) followed by amikacin (95.78%). Resistance to ciprofloxacin was very high (83.10%) (**Table 1**).

### 4. Discussion

The prevalence of ESBL-producing organism is increasing worldwide. In addition resistance to cephalosporins, ESBL-producing organisms are also exhibiting resistance to fluoroquinolones group of drugs limiting further therapeutic options [3].

In this study, out of 75 DDST positive *E. coli*, 71 (94.67%) were confirmed as
ESBL-producer when tested by PCDDT. The result of this study was consistent with the study by Ingviya et al., (2003) [5] in Thailand, who showed that among 100 DDST positive Escherichia coli, 96 (96.0%) Escherichia coli were proved as ESBL-producer by PCDDT.

In this study, 85.92% ESBL-producing Escherichia coli showed high MICs value against ciprofloxacin (2 μg/ml to 128 μg/ml) indicating high level resistance to ciprofloxacin. Similar findings were observed by Hassan et al. [14], who found 85% ESBL-producing Escherichia coli were resistant to ciprofloxacin. This less susceptibility may be due to widespread indiscriminate use, their oral route of administration, easy availability and affordability of ciprofloxacin over the country [14]. Rising MIC values of ciprofloxacin may lead to prolonged treatment, delayed recovery or post-treatment failure. The result of this study was not consistent with the study by Inviya et al. [5], in Thailand, who reported 47% ESBL-producing E. coli were resistant to ciprofloxacin. These findings suggest that sensitivity of ESBL-producing bacteria to ciprofloxacin is gradually decreasing.

About 95.78% ESBL-producing Escherichia coli in this study were sensitive to amikacin. Similar findings were described by Soriozano et al., [15] in Spain and Liao et al., [16] in Taiwan, who found 100% ESBL-producing Escherichia coli were sensitive to amikacin. This result indicates that amikacin can be considered as drug of choice in the treatment of infections caused by ESBL-producing organisms.

In this study, 100% ESBL-producing Escherichia coli were sensitive to imipenem (MIC 0.125 μg/ml to 0.25 μg/ml). Similar findings were observed by Liao et al. [16], Soriozano et al. [15], Ingviya et al. [5], who found 100% sensitivity to imipenem against ESBL-producing Escherichia coli. Carbapenems (e.g. imipenem) are known to be stable against ESBL enzymes and effective in the treatment caused by ESBL-producing bacteria [17].

In conclusion, treatment of choice for infections caused by ESBL-producing organism can be the imipenem and amikacin, as ESBL-producing organism is highly sensitive to these two drugs. ESBL-producing organisms in this study exhibited high resistance to ciprofloxacin. It should be given if they show in vitro susceptibility.

**Contribution to Author**

This work was carried out in collaboration between all authors. Author Jogendra Nath Sarker designed and did the study, collected the data and drafted the manuscript. Authors Shaheda Anwar and Ahmed Abu Saleh supervised the study. Authors Shirin Tarafder drafted and revised the manuscript. Authors S M Ali Ahmed and Hafiza Sultana helped to design the study and to collect data. All authors read and approved the final manuscript.

**Conflicts of Interest**

The authors declare no conflicts of interest regarding the publication of this paper.
References

[1] Bradford, P.A. (2001) Extended-Spectrum β-Lactamases in 21st Century: Characterization, Epidemiology and Detection of This Important Resistance Threat. *Clinical Microbial Reviews, 14*, 933-951. [https://doi.org/10.1128/CMR.14.4.933-951.2001](https://doi.org/10.1128/CMR.14.4.933-951.2001)

[2] Lalitha, M.K. (2004) Manual on Antimicrobial Susceptibility Testing. Department of Microbiology, Christian Medical College Vellore, Tamil Nadu.

[3] Chaudhary, U. and Aggarwal, R. (2004) Extended-Spectrum β-Lactamases (ESBL)—An Emerging Threat to Clinical Therapeutics. *Indian Journal of Medical Microbiology, 22*, 75-80.

[4] Al-Jasser, A.M. (2006) Extended-Spectrum β-Lactamases (ESBL’s): A Global Problem. *Kuwait Medical Journal, 38*, 171-185.

[5] Ingviya, N., Hortiwakul, R., Chayakul, P. and Thamjarungwong, B. (2003) Prevalence and Susceptibility Patterns of *Klebsiella pneumoniae* and *Escherichia coli* Producing Extended-Spectrum β-Lactamases in Songkalanagarind Hospital, Thailand. *Journal of Infectious Diseases and Antimicrobial Agents, 20*, 127-134.

[6] Ahmed, K., Thokar, M.A., Toboli, A.S. and Fomda, B.A. (2010) Extended-Spectrum β-Lactamase Mediated Resistance in *E. coli* in a Tertiary Care Hospital in Kashmir, India. *African Journal of Microbiology Research, 4*, 2720-2728.

[7] Bindayna, K.M., Senok, A.C. and Jamsheer, A.E. (2009) Extended-Spectrum β-Lactamases (ESBL) Producing *Enterobacteriaceae* in Bahrain. *Journal of Infection and Public Health, 2*, 129-135. [https://doi.org/10.1016/j.jiph.2009.04.005](https://doi.org/10.1016/j.jiph.2009.04.005)

[8] Umadevi, S., Kandhakumari, G., Joseph, N.M., Kumar, S., Easow, J.M., Stephen, S., et al. (2011) Prevalence and Antimicrobial Susceptibility Pattern of ESBL-Producing Gram-Negative Bacilli. *Journal of Clinical and Diagnostic Research, 5*, 236-239.

[9] White, G., Acar, J., Anthony, F., Franklin, A., et al. (2001) Antimicrobial Resistance: Standardization and Harmonization of Laboratory Methodologies for the Detection and Quantification of Antimicrobial Resistance. *Revue Scientifique et Technique de L Office International des Epizooties, 20*, 849-858. [https://doi.org/10.20506/rst.20.3.1316](https://doi.org/10.20506/rst.20.3.1316)

[10] Levinson, W. (2006) Antimicrobial Drugs: Resistance. In: Levinson, W.E., Ed., *Review of Medical Microbiology and Immunology*, 9th Edition, McGraw Hill, New York, 85-93.

[11] Chaikittisuk, N. and Munirschoom, A. (2007) Extended-Spectrum β-Lactamases (ESBL) Producing *Klebsiella pneumoniae* in Children at Queen Sirikit National Institute of Child Health. *Journal of Infectious Diseases and Antimicrobial Agents, 24*, 107-115.

[12] Jarlier, V., Nicolas, M.H., Fournier, G. and Philippon, A. (1988) ESBLs Conferring Transferable Resistance to Newer β-Lactam Agents in *Enterobacteriaceae*. Hospital Prevalence and Susceptibility Patterns. *Reviews of Infectious Diseases, 10*, 867-878. [https://doi.org/10.1093/clinids/10.4.867](https://doi.org/10.1093/clinids/10.4.867)

[13] Clinical and Laboratory Standards Institute (2011) Performance Standards for Antimicrobial Susceptibility Testing; 21st Informational Supplement. CLSI Document M100-S21, Wayne.

[14] Hassan, S.A., Jamal, S.A. and Kamal, M. (2011) Occurrence of Multidrug Resistant and ESBL-Producing *E. coli* Causing Urinary Tract Infections. *Journal of Basic and Applied Sciences, 7*, 39-43.

[15] Soriozono, A., Gutierrez, J., Romero, J.M., et al. (2007) Activity in Vitro of Twelve Antibiotics against Clinical Isolates of Extended-Spectrum β-Lactamase Producing
Escherichia coli. Journal of Basic Microbiology, 47, 413-416.
https://doi.org/10.1002/jobm.200710318

[16] Liao, C.H., Sheng, W.H., Wang, J.T., et al. (2006) In Vitro Activities of 16 Antimicrobial Agents against Clinical Isolates of Extended-Spectrum β-Lactamase Producing Escherichia coli and Klebsiella pneumoniae in Two Regional Hospitals in Taiwan. Journal of Microbiology, Immunology and Infection, 39, 59-66.

[17] Kader, A.A. and Kumar, A. (2005) Prevalence and Antimicrobial Susceptibility of Extended-Spectrum β-Lactamase-Producing Escherichia coli and Klebsiella pneumoniae in a General Hospital. Annals of Saudi Medicine, 25, 239-242.
https://doi.org/10.5144/0256-4947.2005.239