Evaluation of extended spectrum beta lactamase enzymes prevalence in clinical isolates of *Escherichia coli*

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Abstract

Resistance to β -lactam antibiotics by gram-negative bacteria, especially *Escherichia coli* (*E. coli*), is a major public health issue worldwide. The predominant resistance mechanism in gram negative bacteria particularly *E. coli* is via the production of extended spectrum beta lactamase (ESBLs) enzymes. In recent years, the prevalence of β -lactamase producing organisms is increased and identification of these isolates by using disk diffusion method and no-one else is not satisfactory. So, this investigation focused on evaluating the prevalence of ESBL enzymes by disk diffusion method and confirmatory test (Combined Disk). Five hundred clinical samples were collected and 200 *E. coli* isolates were detected by standard biochemical tests. To performing initial screening of ESBLs was used from Disk diffusion method on *E. coli* isolates. A confirmation test (Combined Disk method) was performed on isolates of resistant to cephalosporin's indicators. Up to 70% isolates exhibited the Multi Drug Resistance phenotype. In Disk diffusion method, 128(64%) *E. coli* isolates which resistant to ceftazidime and cefotaxime while in Combined Disk, among 128 screened isolates, 115 (89.8%) isolates were detected as ESBLs producers. This survey indicate beta lactamase enzymes are playing a significant role in antibiotic resistance and correct detection of them in phenotypic test by using disk diffusion and combined Disk is essential for accurate recognition of ESBLs.

Introduction

Different strategies are used to protect bacteria from destructive effects of antibiotics.¹ One of the most important resistant mechanisms by bacteria against antibiotics is production of enzymes that hydrolyze the beta lactam ring of antibiotics. The action of these antibiotics is via the inhibition of cell wall synthesis and bacterial cell division.² Extended spectrum beta lactamase (ESBLs) are specific types of beta lactamases with particular mutation, making them capable of destroying expanded cephalosporin (cefotaxime, ceftazidime) and aztreonam, but don't have activity against carbapenems (imipenem), cephamycins (cephoxitin) and beta lactam inhibitor (clavulanic acid, sulbactam).³ On the other hand, development of novel antibiotics and promotion of their use in the treatment of bacterial infectious diseases led to the appearance of newer enzyme classes in beta lactamase super family particularly AmpC.⁴ ESBLs enzymes were discovered in early 1980 and these are classified based on four functional groups: A,B,C and D that ESBLs is placed in A groups and derived from principal beta lactamases (TEM-1, TEM-2 and SHV-1) by substitution one or more amino acids in their catalytic sites.⁵ Among Enterobacteriaceae, *Escherichia coli* (*E. coli*) and *Klebsiella* spp. are more known to produce ESBLs.⁶ *E. coli* is the most commonly encountered gram negative pathogen in nosocomial infections.⁷ Recently, the occurrence of ESBLs is rising worldwide; owing to identification of these strains in clinical laboratories to detect this resistance is very important.⁸ According to the recommendation of the Clinical and Laboratory Standards Institute (CLSI),⁹ one of the current methods for detection of ESBLs is an initial screening for the CLSI recommended cephalosporin's indicators especially ceftazidime and cefotaxime, subsequently performing of confirmatory tests with clavulanic acid (as one inhibitor of ESBLs). In this test, if zone of inhibition in the presence of clavulanic acid in compare absence clavulanic acid is increased, those organisms are recognized as a ESBLs producer.^{10,11} Complete detection of β -lactamase enzymes is essential for resistance control and successful treatment via the appropriate prescription of β -lactam drugs. Therefore, we focused this study to determine the incidence of ESBLs producing strains in clinical isolates.

Materials and Methods

Bacterial strains

Five hundred clinical samples were collected

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Contributions: RB, microbiological test; JFM, molecular test results analysis; HMA, samples collection and primer design; AS, samples collection and molecular test; MMSD, upervising the project.

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from Tehran hospitals (kid's medical center, Shariati, Mofid, Ali Asghar, Eghbal, Hakim, Valiasr, Khatamolanbia and Emam Khomeini) as of the following sources: urine, stool, blood, wound and other clinical samples of sick people. Two hundred *E. coli* isolates were detected by standard biochemical tests (IMVIC) by using TSI agar, SIM agar, Simmons Citrate agar, MR-VP medium and Urea media. Owing to store, all strain of *E. coli* isolated were cultured in skim milk and were stocked up at -70°C prior for testing.

Antimicrobial susceptibility testing for screening of extended spectrum beta lactamase

To performing initial screening of ESBLs was used from Disk diffusion method⁹ on *E. coli* isolates. In this method, after the preparation of Muller-Hinton agar plates, microbial suspensions corresponding with concentration of 0.5 McFarland were completely distributed on the surface of plates. The following antibiotics and concentration were used: cefotaxime (30 μ g), ceftazidime (30 μ g), gentamicin (10 μ g), amoxicillin (30 μ g), imipenem (10 μ g), nalidixic acid (30 μ g), streptomycin (10 μ g), cotrimoxazole (1.25 μ g), ciprofloxacin (5 μ g) and chloramphenicol (30 μ g) (Mast Diagnostics Ltd., Bootle, Merseyside, UK). After 24 h incubation of plates at 37°C, using a

ruler, the zone of inhibition around each disk was measured and then compared with the standards of CLSI, followed by reported as resistant, intermediate or sensitive.^{9,12} A confirmation test (Combined Disk method)⁹ was performed on isolates of resistant to

Figure 1. The frequency and source of *E. coli* isolates collected from Tehran hospitals during 6 months.

= Imipenem
CIP = Ciprofloxacin
GM = Gentamicin
C = Chloramphenicol
S = Streptomycin
CTX = Cefotaxime
CAZ = Ceftazidime
TS = Cotrimoxazole
NA = Nalidixic acid
A = Amoxicillin

Figure 2. Pattern of resistance among 200 *E. coli* isolates to 10 antimicrobial agents.

Figure 3. Combined Disk assay for detection of extended spectrum beta lactamase. CTX, Cefotaxime; CAZ, Ceftazidime; CV, clavulanic acid.

cephalosporin's indicators which used from Ceftazidime (30 µg), Ceftazidime/Clavulanate (30/10 µg), Cefotaxime (30 µg) and Cefotaxime/Clavulanate (30/10 µg) (Mast Diagnostics Ltd.). After 24 h incubation of plates at 37°C, ESBLs production was confirmed by ≥ 5 mm increase in the zone of inhibition in the presence of clavulanic acid compare to the absence of clavulanic acid.^{13,14}

Results

Two hundred clinical isolates of *E. coli* were collected during a period of 6 months (Figure 1). Resistant pattern of 200 *E. coli* isolates to 10 antimicrobial agents are shown in Figure 2. Most of the isolates indicated high resistance to oxyiminocephalosporins, while they remained susceptible to imipenem. Up to 70% isolates exhibited the MDR (Multi Drug Resistance) phenotype.

In Disk diffusion method, 128(64%) *E. coli* isolates which resistant to ceftazidime and cefotaxime were selected for possible positive ESBLs and followed by Combined Disk assay. In Combined Disk, among 128 screened isolates, 115(89.8%) isolates were detected as ESBLs producers (Figure 3). β -lactamase-producing *E. coli* isolates was more common in urine samples (80%).

Discussion

This study was conducted in Tehran hospitals such as kid's medical center, Shariati, Mofid, Ali Asghar, Eghbal, Hakim, Valiasr, Khatamolanbia and Emam Khomeini. Among 500 clinical samples from sick people, 200 *E. coli* were isolates. ESBL screening and confirmation were performed by Disk diffusion method and Combined Disk assay, respectively. *E. coli* ESBLs positive culture cases were 89.8% alternatively. The resistant rate of *E. coli* to cephalosporin's indicators in this study higher than that (32.11%) in the study of Nakhaei et al which reported from Iran.¹⁵ The results indicate that the ESBLs prevalence in community acquired infections particularly in *E. coli* is rising in Iran. In recent years, studies were reported that significant proportion of Enterobacteriaceae isolates showed multidrug resistance against extended cephalosporins and aztreonam, that ESBLs production have the predominant role in this field.¹⁶ So, correct detection of them led to prevent from failure treatment and suitable prescription drugs.

The high prevalence of ESBL suggests that diagnostic laboratories should be better than phenotypic screen for ESBLs with ceftazidime as well as cefotaxime;¹⁷ they should still per-

form clavulanate synergy tests on resistant isolates, but ESBLs may not be detected by standard laboratory methods because susceptibility results for cephalosporins may fall within the sensitive category and reported as false negative ESBLs which resulting from another factors such as AmpC that cover the effect of ESBL enzymes via creating resistance to clavulanate.^{16,18}

In this survey among of 128 (64%) screened *E. coli* isolates in Disk diffusion method, only 115 (89.8%) isolate were selected for ESBLs producers by Combined Disk according to the CLSI.⁹ Negative ESBLs in other isolates (10.2%) could be resulting other factors such as novel beta lactamase enzymes.

Conclusions

Today there are many β -lactam antibiotics and many more β -lactamase enzymes, including extended spectrum β -lactamases (ESBLs) and AmpC β -lactamases¹⁹ that make a many problem in detection of ESBLs. So, this investigation suggests using other methods including inhibitors of AmpC in confirmatory test for correct detection of ESBLs.

References

- Li Q, Lee YJ, Castillo R, et al. NB2001, a novel antibacterial agent with broad-spectrum activity and enhanced potency against β -lactamase-producing strains. *Antimicrob Agents Chemother* 2002;46: 1262-8.
- Perilli M, Celenza G, Santis DF, et al. E240V substitution increases catalytic efficiency toward ceftazidime in a New natural TEM-type extended-spectrum β -lactamase, TEM-149, from *Enterobacter aerogenes* and *Serratia marcescens* clinical isolates. *Antimicrob Agents Chemother* 2008;52:915-9.
- Tenover FC, Raney PM, Williams PP, et al. Evaluation of the NCCLS extended-spectrum β -lactamase confirmation methods for *Escherichia coli* with isolates collected during project ICARE. *J Clin Microbiol* 2003;41:3142-6.
- Bush K, Jacoby GA, Medeiros AA. A functional classification scheme for β -lactamases and its correlation with molecular structure. *Antimicrob Agents Chemother* 1995;39:1211-33.
- Datta N, Kontomichalou P. Penicillinase synthesis controlled by infectious R factors in *Enterobacteriaceae*. *Nature* 1965;208: 239-41.
- Eagye JK, Kuti LJ, Nicolau PD. Pharmacology

- dynamic Comparison of the Carbapenems Against *E. coli* and *Klebsiella* spp. Containing Extended spectrum β -lactamases. *Am J Infect Dis* 2005;1:149-55.
7. Randrianirina F, Vaillant L, Ramarokoto EC, et al. Antimicrobial resistance in pathogens causing nosocomial infections in surgery and intensive care wards in Antananarivo, Madagascar. *J Infect Dev Ctries* 2010;4:74-82.
 8. Suvorov M, Vakulenko SB, Mobashery S. Cytoplasmic-membrane anchoring of a class A β -lactamase and its capacity in manifesting antibiotic resistance. *Antimicrob Agents Chemother* 2007;51:2937-42.
 9. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing: Fifteenth Informational Supplement. 2005. M100-S15 Vol. 25 No. 1.
 10. Stürenburg E, Mack D. Extended-spectrum β -lactamases: implications for the clinical microbiology laboratory, therapy, and infection control. *J Infect* 2003;47:273-95.
 11. Pitout JDD, Laupland KB. Extended-spectrum β -lactamase-producing Enterobacteriaceae: an emerging public-health concern. *Lancet Infect Dis* 2008;8:159-66.
 12. Netzel TC, Jindani I, Hanson N, et al. The AmpC inhibitor, Syn2190, can be used to reveal extended-spectrum β -lactamases in *Escherichia coli*. *Diagn Microbiol Infect Dis* 2007;58:345-8.
 13. Song W, Bae K, Lee YN, et al. Detection of extended-spectrum β -lactamases by using Boronic acid as an AmpC β -lactamase inhibitor in clinical isolates of *Klebsiella* spp. and *Escherichia coli*. *J Clin Microbiol* 2007;45:1180-4.
 14. Deshpande LM, Jones RN, Fritsche TR, Sader HS. Occurrence of plasmidic AmpC type β -lactamase-mediated resistance in *Escherichia coli*: report from the SENTRY Antimicrobial Surveillance Program (North America, 2004). *Int J Antimicrob Agents* 2006;28:578-81.
 15. Nakhaei MM, Moshrefi SH. Determine the pattern of antibiotic resistance urine *E. coli* isolates and survey the prevalence of broad spectrum β -lactamases. *J Med Centers Sabzevar* 2009;4:228-33.
 16. Hawser PS, Bouchillon KS, Hoban JD, et al. Emergence of High Levels of Extended-Spectrum β -Lactamase-Producing Gram-Negative Bacilli in the Asia-Pacific Region: data from the study for monitoring antimicrobial resistance trends (SMART) Program, 2007. *Antimicrob Agents Chemother* 2009;53:3280-4.
 17. Rawat D, Nair D. Extended-spectrum β -lactamases in Gram Negative Bacteria. *J Glob Infect Dis* 2010;2:263-74.
 18. Liu G, Ling BD, Zeng Y, et al. Molecular characterization of Extended-Spectrum β -lactamases Produced by clinical isolates of *Enterobacter cloacae* from a Teaching Hospital in china. *Jpn Infect Dis* 2008;61:286-9.
 19. Jeong SH, Song W, Kim JS, et al. Broth Microdilution Method To Detect Extended-Spectrum β -Lactamases and AmpC β -Lactamases in Enterobacteriaceae Isolates by Use of Clavulanic Acid and Boronic Acid as Inhibitors. *J Clin Microbiol* 2009;47:3409-12.