Prevalence and antibiotic resistance pattern of extended-spectrum beta-lactamase-producing
Escherichia coli in clinical specimens

Kiana Shirani¹, Elahe Seydayi¹, Kiarash Salimi Boroujeni²
¹Isfahan Infectious Diseases Research Center, Isfahan University of Medical Sciences, Isfahan, Iran, ²Faculty of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran

Background: Extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae seem to have an extended antibiotic resistance, but have different resistance patterns throughout different sites and regions. This study aimed to evaluate the antibiotic resistance pattern of ESBL-producing Escherichia coli. Materials and Methods: One hundred swab samples from patients hospitalized due to a clinical suspicion of any kind of infection (with manifestations such as fever, leukocytosis, and an active urinalysis result) were processed in Alzahra Microbiology Laboratory, Isfahan, Iran. Isolated E. coli were cultured on Mueller–Hinton agar and antibiotic susceptibility was tested by Kirby–Bauer disk diffusion method following the Clinical and Laboratory Standard Institute 2017 guidelines. Results: ESBL-producing samples had higher antibiotic resistance rates than ESBL-non-producing samples: ceftriaxone (58.8% vs. 27.3%), cefotaxime (73.5% vs. 30.3%), cefixime (76.5% vs. 33.3%), cefixime (79.4% vs. 40.9%), and cefpodoxime (73.5% vs. 53%), except for carbenicillin (29.4% vs. 48.5%). Imipenem and meropenem were the least resisted antibiotics in ESBL-producing samples (5.9% and 11.8%). Conclusion: ESBL-producing Enterobacteriaceae have a high resistance rate to third-generation cephalosporins and high susceptibility to imipenem and meropenem.

Key words: Bacterial, beta-lactamases, drug resistance, Escherichia coli

How to cite this article: Shirani K, Seydayi E, Boroujeni KS. Prevalence and antibiotic resistance pattern of extended-spectrum beta-lactamase-producing Escherichia coli in clinical specimens. J Res Med Sci 2019;24:103.

INTRODUCTION

One of the most important mechanisms of bacteria against antibiotics is the production of enzymes destroying β-lactam ring in the antibiotics structure. Extended-spectrum β-lactamase (ESBL) is an important group of β-lactamases.[1] Escherichia coli is the most prevalent and hence the most important multidrug-resistant Gram-negative infection, especially in patients with urinary tract infection (UTI).[2,3] Throughout the recent century, ESBL-producing Enterobacteriaceae have been introduced in the literature.[4] ESBL-producing E. coli has been isolated in community and nosocomial settings as well.[5] This might be a result of extensive antibiotic usage and can cause antibiotic resistance in human pathogens. Infection with ESBL-producing E. coli has an ascending trend of growth in both community and hospital infections in Iran.[6–8]

Sufficient identification of ESBL-producing strains is essential to make an appropriate choice of antimicrobial regimen and evaluation strategy.[9] Because no comprehensive studies in the territory of ESBL-producing E. coli in Iran are available, we aimed to evaluate the prevalence and antibiotic resistance pattern of ESBL-producing E. coli in clinical specimens.

MATERIALS AND METHODS

Study design and target group
Throughout a cross-sectional study, we evaluated clinical specimens from hospitalized patients in Isfahan Alzahra Hospital, Center of Iran, from August to December 2015. Four milliliters of midstream
Shirani, et al.: Antibiotic resistance pattern in E. coli with ESBL
Journal of Research in Medical Sciences| 2019 |

urine was collected from each patient into a sterile tube. Samples were then transported to the hospital laboratory as soon as possible. Patients were instructed properly for the means of sampling. Sources of the samples varied throughout the patients, in accordance with their symptoms, practitioners’ clinical suspicion, and the standard diagnostic guidelines (with blood [24%], urine [44%], abscess [3%], CSF [4%], sputum [5%], rectal swab [3%], perianal swab [3%], and skin swab [13%], differing based on the patients’ manifestations).

**Laboratory assessment and extended-spectrum β-lactamase detection**

Two hours after the collection, 100 swab samples, isolated from urine specimen of patients hospitalized due to various reasons with a clinical suspicion of any kind of infection (fever, leucocytosis), were streaked directly on eosin methylene blue agar, MacConkey agar, and blood agar plates. Such plates were incubated at 37°C aerobically, and after overnight incubation, they were assessed for E. coli growth. E. coli existence was proved by their colony morphology, Gram staining characteristics, biochemical tests of glucose fermentation, Voges–Proskauer reaction (acetyl methyl carbinol production from dextrose) on the Triple Sugar Iron agar, gas producing, lactose metabolism, production of indole from tryptophan, sulfide-indole-motility, and methyl red Voges–Proskauer.

Isolated E. coli were cultured on Mueller–Hinton agar (MHA), and antibiotic susceptibility was tested by Kirby–Bauer disk diffusion method after the Clinical and Laboratory Standard Institute (CLSI) guidelines, 2017. Below is the list of drug concentrations used for disc diffusion testing: ceftazidime (30 μg; inhibition zone (IZ) size equal or smaller than 22 mm), amikacin (30 μg; IZ ≤ 22 mm), ampicillin (10 μg), gentamicin (10 μg; IZ ≤ 22 mm), cefixime (5 μg), cefotaxime (30 μg; IZ ≤ 22 mm), amoxicillin/clavulanic acid (30 μg), ceftriaxone (30 μg; IZ ≤ 22 mm), ciprofloxacin (5 μg), cotrimoxazole (23.75 μg sulfamethoxazole/1.25 μg trimethoprim), ceftizoxime (30 μg), imipenem (10 μg), meropenem (30 μg), nalidixic acid (30 μg), gentamicin (10 μg), carbenicillin (100 μg), and cefpodoxime (30 μg; IZ ≤ 17 mm).

Isolates showing IZs less than the values stated above were interpreted as screening positive for ESBL production. Only E. coli were screened for ESBL production.

For ESBL confirmation, 2–3 colonies of the organisms were suspended in 0.5 ml of sterile broth and the turbidity matched to 0.5 McFarland. Using a sterile cotton swab, the broth culture was uniformly swabbed on MHA. All the E. coli isolates resistant to at least ceftazidime, ceftriaxone, and/or cefotaxime were tested for confirmation using cefotaxime–clavulanic acid (30 μg + 10 μg), cefotaxime (30 μg), ceftazidime–clavulanic acid (30 μg + 10 μg), and ceftazidime (30 μg) combination disks. The tests were interpreted according to the most recent CLSI guidelines (2017), and a difference of 5 mm between IZ of a single disk and in combination with clavulanic acid (inhibitor) was confirmed to be produced by an ESBL-positive isolate.

**Data analysis**

Statistical analysis of data was performed using SPSS 22.0 software. To compare qualitative variables between groups, Chi-square test was performed. The normal distribution of all studied parameters was checked with Kolmogorov–Smirnov test. Student’s t-test was used for variables which were distributed in a normal way, besides Mann–Whitney and Wilcoxon tests were performed for variables that have not normal distribution. Two-tailed P < 0.05 was considered statistically significant.

**RESULTS**

The results of the study showed that ESBL-producing E. coli was found in 34% of all samples (ergo 34 ESBL screening-positive samples). ESBL-producing samples had higher antibiotic resistance rate to third-generation cephalosporins than ESBL-non-producing samples such as ceftriaxone (58.8% vs. 27.3%, P < 0.001), cefotaxime (73.5% vs. 30.3%, P < 0.001), ceftizoxime (76.5% vs. 33.3%, P < 0.001), cefixime (79.4% vs. 40.9%, P < 0.001), and cefpodoxime (73.5% vs. 53%, P = 0.045). On the other hand, carbenicillin in ESBL-producing samples had lower antibiotic resistance rate than ESBL-non-producing samples (29.4% vs. 48.5%, P = 0.031), which is a rather strange finding. Furthermore, we found that imipenem and meropenem had the lowest antibiotic resistance rate in ESBL-producing samples (5.9% and 11.8%) [Tables 1 and 2].

**Table 1: Demographic characteristics of patients and studied variables on account of extended-spectrum β-lactamase production**

| Variables | ESBL Positive (%) | ESBL Negative (%) | P |
|-----------|-------------------|-------------------|---|
| Age (years) |                     |                   |   |
| Male (53) | 46.35±12.97       | 45.93±11.8        | 0.873 |
| Female (47) | 15 (44.1)       | 38 (57.6)        | 0.201 |
| Clinic sample |                   |                   |   |
| Blood (24) | 7 (20.6)          | 17 (52.8)        | 0.176 |
| Urine (44) | 14 (41.2)         | 31 (47)          |   |
| Abscess (3) | 0                 | 3 (4.5)         |   |
| CSF (4) | 2 (5.9)           | 2 (5)            |   |
| Sputum (5) | 1 (2.9)          | 4 (6.1)         |   |
| Rectal swab (3) | 2 (6.9) | 1 (3.5)   |   |
| Perianal swab (3) | 0     | 3 (4.5)   |   |
| Skin swab (13) | 8 (23.5)        | 5 (7.6)        |   |

ESBL=Extended-spectrum β-lactamase
Shirani, et al.: Antibiotic resistance pattern in E. coli with ESBL
Journal of Research in Medical Sciences | 2019 |

Table 2: Antibiotic susceptibility patterns on account of extended-spectrum β-lactamase production

| Antibiotics            | Sensitive (%) | Intermediate (%) | Resistance (%) | ESBL (%)        |
|------------------------|---------------|------------------|----------------|----------------|
|                        | Positive (%)  |                   |                | ESBL (%)       |
|                        |               | Intermediate    |                | ESBL (%)       |
|                        |               | Resistance      |                | ESBL (%)       |
|                        |               | Positive (%)    | ESBL (%)       |
|                        |               | Intermediate    | ESBL (%)       |
|                        |               | Resistance      | ESBL (%)       |
|                        |               | Positive (%)    | ESBL (%)       |
|                        |               | Intermediate    | ESBL (%)       |
|                        |               | Resistance      | ESBL (%)       |
|                        |               | Positive (%)    | ESBL (%)       |
|                        |               | Intermediate    | ESBL (%)       |
|                        |               | Resistance      | ESBL (%)       |
|                        |               | Positive (%)    | ESBL (%)       |
|                        |               | Intermediate    | ESBL (%)       |
|                        |               | Resistance      | ESBL (%)       |
|                        |               | Positive (%)    | ESBL (%)       |
|                        |               | Intermediate    | ESBL (%)       |
|                        |               | Resistance      | ESBL (%)       |
|                        |               | Positive (%)    | ESBL (%)       |
|                        |               | Intermediate    | ESBL (%)       |
|                        |               | Resistance      | ESBL (%)       |
|                        |               | Positive (%)    | ESBL (%)       |
|                        |               | Intermediate    | ESBL (%)       |
|                        |               | Resistance      | ESBL (%)       |
|                        |               | Positive (%)    | ESBL (%)       |
|                        |               | Intermediate    | ESBL (%)       |
|                        |               | Resistance      | ESBL (%)       |
|                        |               | Positive (%)    | ESBL (%)       |
|                        |               | Intermediate    | ESBL (%)       |
|                        |               | Resistance      | ESBL (%)       |
|                        |               | Positive (%)    | ESBL (%)       |
|                        |               | Intermediate    | ESBL (%)       |
|                        |               | Resistance      | ESBL (%)       |
|                        |               | Positive (%)    | ESBL (%)       |
|                        |               | Intermediate    | ESBL (%)       |
|                        |               | Resistance      | ESBL (%)       |
|                        |               | Positive (%)    | ESBL (%)       |
|                        |               | Intermediate    | ESBL (%)       |
|                        |               | Resistance      | ESBL (%)       |
|                        |               | Positive (%)    | ESBL (%)       |
|                        |               | Intermediate    | ESBL (%)       |
|                        |               | Resistance      | ESBL (%)       |
|                        |               | Positive (%)    | ESBL (%)       |
|                        |               | Intermediate    | ESBL (%)       |
|                        |               | Resistance      | ESBL (%)       |
|                        |               | Positive (%)    | ESBL (%)       |
|                        |               | Intermediate    | ESBL (%)       |
|                        |               | Resistance      | ESBL (%)       |
|                        |               | Positive (%)    | ESBL (%)       |
|                        |               | Intermediate    | ESBL (%)       |
|                        |               | Resistance      | ESBL (%)       |
|                        |               | Positive (%)    | ESBL (%)       |
|                        |               | Intermediate    | ESBL (%)       |
|                        |               | Resistance      | ESBL (%)       |
|                        |               | Positive (%)    | ESBL (%)       |
|                        |               | Intermediate    | ESBL (%)       |
|                        |               | Resistance      | ESBL (%)       |
|                        |               | Positive (%)    | ESBL (%)       |
|                        |               | Intermediate    | ESBL (%)       |
|                        |               | Resistance      | ESBL (%)       |
|                        |               | Positive (%)    | ESBL (%)       |
|                        |               | Intermediate    | ESBL (%)       |
|                        |               | Resistance      | ESBL (%)       |
|                        |               | Positive (%)    | ESBL (%)       |
|                        |               | Intermediate    | ESBL (%)       |
|                        |               | Resistance      | ESBL (%)       |
|                        |               | Positive (%)    | ESBL (%)       |
|                        |               | Intermediate    | ESBL (%)       |
|                        |               | Resistance      | ESBL (%)       |
|                        |               | Positive (%)    | ESBL (%)       |
|                        |               | Intermediate    | ESBL (%)       |
|                        |               | Resistance      | ESBL (%)       |
|                        |               | Positive (%)    | ESBL (%)       |
|                        |               | Intermediate    | ESBL (%)       |
|                        |               | Resistance      | ESBL (%)       |
|                        |               | Positive (%)    | ESBL (%)       |
|                        |               | Intermediate    | ESBL (%)       |
|                        |               | Resistance      | ESBL (%)       |
|                        |               | Positive (%)    | ESBL (%)       |
|                        |               | Intermediate    | ESBL (%)       |
|                        |               | Resistance      | ESBL (%)       |
|                        |               | Positive (%)    | ESBL (%)       |
|                        |               | Intermediate    | ESBL (%)       |
|                        |               | Resistance      | ESBL (%)       |
|                        |               | Positive (%)    | ESBL (%)       |
|                        |               | Intermediate    | ESBL (%)       |
|                        |               | Resistance      | ESBL (%)       |
|                        |               | Positive (%)    | ESBL (%)       |
|                        |               | Intermediate    | ESBL (%)       |
|                        |               | Resistance      | ESBL (%)       |
|                        |               | Positive (%)    | ESBL (%)       |
|                        |               | Intermediate    | ESBL (%)       |
|                        |               | Resistance      | ESBL (%)       |
|                        |               | Positive (%)    | ESBL (%)       |
|                        |               | Intermediate    | ESBL (%)       |
|                        |               | Resistance      | ESBL (%)       |
|                        |               | Positive (%)    | ESBL (%)       |
|                        |               | Intermediate    | ESBL (%)       |
|                        |               | Resistance      | ESBL (%)       |
|                        |               | Positive (%)    | ESBL (%)       |
|                        |               | Intermediate    | ESBL (%)       |
|                        |               | Resistance      | ESBL (%)       |
|                        |               | Positive (%)    | ESBL (%)       |
|                        |               | Intermediate    | ESBL (%)       |
|                        |               | Resistance      | ESBL (%)       |
|                        |               | Positive (%)    | ESBL (%)       |
|                        |               | Intermediate    | ESBL (%)       |
|                        |               | Resistance      | ESBL (%)       |
|                        |               | Positive (%)    | ESBL (%)       |
|                        |               | Intermediate    | ESBL (%)       |
|                        |               | Resistance      | ESBL (%)       |
|                        |               | Positive (%)    | ESBL (%)       |
|                        |               | Intermediate    | ESBL (%)       |
|                        |               | Resistance      | ESBL (%)       |
|                        |               | Positive (%)    | ESBL (%)       |
|                        |               | Intermediate    | ESBL (%)       |
|                        |               | Resistance      | ESBL (%)       |
|                        |               | Positive (%)    | ESBL (%)       |
|                        |               | Intermediate    | ESBL (%)       |
|                        |               | Resistance      | ESBL (%)       |
|                        |               | Positive (%)    | ESBL (%)       |
|                        |               | Intermediate    | ESBL (%)       |
|                        |               | Resistance      | ESBL (%)       |
|                        |               | Positive (%)    | ESBL (%)       |
|                        |               | Intermediate    | ESBL (%)       |
|                        |               | Resistance      | ESBL (%)       |
|                        |               | Positive (%)    | ESBL (%)       |
|                        |               | Intermediate    | ESBL (%)       |
|                        |               | Resistance      | ESBL (%)       |
|                        |               | Positive (%)    | ESBL (%)       |
|                        |               | Intermediate    | ESBL (%)       |
|                        |               | Resistance      | ESBL (%)       |
|                        |               | Positive (%)    | ESBL (%)       |
|                        |               | Intermediate    | ESBL (%)       |
|                        |               | Resistance      | ESBL (%)       |
|                        |               | Positive (%)    | ESBL (%)       |
|                        |               | Intermediate    | ESBL (%)       |
|                        |               | Resistance      | ESBL (%)       |
|                        |               | Positive (%)    | ESBL (%)       |
|                        |               | Intermediate    | ESBL (%)       |
|                        |               | Resistance      | ESBL (%)       |
|                        |               | Positive (%)    | ESBL (%)       |
|                        |               | Intermediate    | ESBL (%)       |
|                        |               | Resistance      | ESBL (%)       |

DISCUSSION

The present piece of research focused solely on the prevalence and antibiotic resistance pattern of ESBL-producing E. coli due to shortage of the project budget.

We found that the prevalence of ESBL-producing bacteria in clinical samples of the hospital was 34%. This is a completely high amount for such a prevalent microorganism which would be really catastrophic in the treatment approaches. This value has been reported in lower amounts in some of the other studies,[13-17] whereas other studies reported higher prevalence as compared to our results.[18,19] As reported in a cross-sectional study by Mihankhah et al., E. coli is among the most prevalent Gram-negative specimens obtained from clinical samples of UTIs in Iran with 37.8% of the whole.[20]

ESBLs are enzymes destroying β-lactam ring in the antibiotic structure, such as monobactams (e.g., aztreonam), third-generation cephalosporins (e.g., ceftriaxone, ceftazidime, and cefotaxime), and carbapenems (e.g., imipenem, meropenem, and ertapenem), but not the cephemycins (e.g., cefoxitin and cefotetan).[21] Such enzymes are sensitive to β-lactamase inhibitors (clavulanic acid, sulbactam, and tazobactam).[22] Bacterial resistance has increased during the recent decades.[23,24] As our statistical data witness, although third-generation cephalosporins are strong and widely used antibiotics, there is a high rate of resistance and they are not a good choice. The most prominent sensitivity it is to imipenem and meropenem and they are better choices. We recommend performing antibiogram in hospital-admitted UTI patients and select the best choice of antibiotics.

CONCLUSION

Our results showed high prevalence of ESBL in hospital samples in Isfahan, Iran. Because Alzahra Hospital is a major and characteristic hospital laboratory dealing specifically with exceptional patients, the conduction of this study in that specific laboratory setting in Isfahan should interest readers from clinical and epidemiological perspective. Our data confirmed that ESBL had high resistance rate to third generation of cephalosporins and high susceptibility to imipenem and meropenem. These findings suggest further studies in this field.

Acknowledgments

This article was carried out as a doctoral dissertation project under the supervision of Isfahan University of Medical Sciences, with the ethical code: IR.MUI.REC.1394.3.719.

Financial support and sponsorship

Isfahan University of Medical Sciences.

Conflicts for interest

There are no conflicts for interest.

REFERENCES

1. Thirapanmet hee K. Extended spectrum β-lactamases: Critical tools of bacterial resistance. Mahidol Univ J Pharm Sci 2012;39:1-8.
2. Hryniecz K, Szczyka K, Sulikowska A, Jankowski K,
Betlejewska K, Hryniewicz W. Antibiotic susceptibility of bacterial strains isolated from urinary tract infections in Poland. J Antimicrob Chemother 2001;47:773-80.

3. Wilson AP, Livermore DM, Otter JA, Warren RE, Jenks P, Enoch DA, et al. Prevention and control of multi-drug-resistant gram-negative bacteria: Recommendations from a joint working party. J Hosp Infect 2016;92 Suppl 1:S1-44.

4. Zahar JR, Lortholary O, Martin C, Potel G, Plesiat P, Nordmann P. Addressing the challenge of extended-spectrum beta-lactamases. Curr Opin Investig Drugs 2009;10:172-80.

5. Chen YH, Hsueh PR, Badal RE, Hawser SP, Hoban DJ, Bouchillon SK, et al. Antimicrobial susceptibility profiles of aerobic and facultative gram-negative bacilli isolated from patients with intra-abdominal infections in the Asia-Pacific Region according to currently established susceptibility interpretive criteria. J Infect 2011;62:280-91.

6. Behroozi A, Rahbar M, Jalil V. Frequency of extended spectrum beta-lactamase (ESBLs) producing Escherichia coli and Klebsiella pneumoniae isolated from urine in an Iranian 1000-bed tertiary care hospital. Afr J Microbiol Res 2010;4:881-4.

7. Mehran H, Rahbar M. Prevalence of extended-spectrum beta-lactamase-producing Escherichia coli in a tertiary care hospital in Tehran, Iran. Int J Antimicrob Agents 2008;31:147-51.

8. Khanfar HS, Bindayna KM, Senok AC, Botta GA. Extended spectrum beta-lactamases (ESBL) in Escherichia coli and Klebsiella pneumoniae: Trends in the hospital and community settings. J Infect Dev Ctries 2009;3:295-9.

9. Cohen Stuart J, Dierikx C, Al Naiemi N, Karczmarek A, Van Hoek AH, Vos P, et al. Rapid detection of TEM, SHV and CTX-M extended-spectrum beta-lactamases in Enterobacteriaceae using ligation-mediated amplification with microarray analysis. J Antimicrob Chemother 2010;65:1377-81.

10. Garcia LS. Clinical Microbiology Procedures Handbook. American Society for Microbiology Press; 2010.

11. Baron EJ, Thomson RB. Specimen collection, transport, and processing: Bacteriology. In: Manual of Clinical Microbiology. 10th ed. American Society of Microbiology; 2011. p. 226-71.

12. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing of Anaerobic Bacteria: Informational Supplement 27th ed (2017). CLSI Document M100). Clinical and Laboratory Standards Institute; 2017.

13. Valenza G, Nickel S, Pfeifer Y, Pietsch M, Voigtländer E, Lehner-Reindl V, et al. Prevalence and genetic diversity of extended-spectrum β-lactamase (ESBL)-producing Escherichia coli in nursing homes in Bavaria, Germany. Vet Microbiol 2017;200:138-41.

14. Yadav KK, Adhikari N, Khadka R, Pant AD, Shah B. Multidrug resistant Enterobacteriaceae and extended spectrum β-lactamase producing Escherichia coli: A cross-sectional study in national kidney center, Nepal. Antimicrob Resist Infect Control 2015;4:42.

15. Čornejová T, Venglovsky J, Gregova G, Kmetova M, Kmet V. Extended spectrum beta-lactamases in Escherichia coli from municipal wastewater. Ann Agric Environ Med 2015;22:447-50.

16. Ghorbani-Dalini S, Kargar M, Doosti A, Abbasi P, Sarshar M. Molecular epidemiology of ESBL genes and multi-drug resistance in diarrheagenic Escherichia coli strains isolated from adults in Iran. Iran J Pharm Res 2015;14:1257-62.

17. Kumar MR, Arunagirinathan N, Vignesh R, Balakrishnan P, Solomon S, Sunil SS. Ertapenem for multiple β-lactamases producing gram-negative bacteria causing urinary tract infections in HIV patients. J Res Med Sci 2017;22:69.

18. Anago E, Ayi-Fanou L, Akpovi CD, Hounkpe WB, Agassounon-Djikpo Tchibozo M, Bankole HS, et al. Antibiotic resistance and genotype of beta-lactamase producing Escherichia coli in nosocomial infections in Cotonou, Benin. Ann Clin Microbiol Antimicrob 2015;14:5.

19. Arabi H, Pakzad I, Nasrollahi A, Hosainzadegan H, Azizi Jallian F, Taherikalani M, et al. Sulfonamide resistance genes (sul) M in extended spectrum beta lactamase (ESBL) and non-ESBL producing Escherichia coli isolated from Iranian hospitals. Jundishapur J Microbiol 2015;8:e19961.

20. Mihankhah A, Khoshbakht R, Raeisi M, Raeisi V. Prevalence and antibiotic resistance pattern of bacteria isolated from urinary tract infections in Northern Iran. J Res Med Sci 2017;22:108.

21. Bradford PA. Extended-spectrum beta-lactamases in the 21st century: Characterization, epidemiology, and detection of this important resistance threat. Clin Microbiol Rev 2001;14:933-51, table of contents.

22. Mukherjee M, Basu S, Mukherjee SK, Majumder M. Multidrug-resistance and extended spectrum beta-lactamase production in uropathogenic E. coli which were isolated from hospitalized patients in Kolkata, India. J Clin Diagn Res 2013;7:449-53.

23. Gupta K, Hooton TM, Stamm WE. Increasing antimicrobial resistance and the management of uncomplicated community-acquired urinary tract infections. Ann Intern Med 2001;135:41-50.

24. Levy SB, Marshall B. Antibacterial resistance worldwide: Causes, challenges and responses. Nat Med 2004;10:SI22-9.