

Prevalence of Extended Spectrum β -Lactamase Producing *Klebsiella Pneumoniae* Isolates in A Tertiary Care Hospital in Bangladesh

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Abstract

Background: Extended-spectrum β lactamases (ESBLs) continue to be a major challenge in clinical setups world over, conferring resistance to the expanded-spectrum cephalosporins. **Objectives:** The aim of this study was to determine the prevalence of extended spectrum β -lactamase (ESBL) in strains of *Klebsiella pneumoniae* isolated from different clinical specimens in Dhaka Medical College Hospital, Dhaka, Bangladesh. **Methodology:** This cross sectional study was carried out at the Department of Microbiology of Dhaka Medical College, Dhaka, Bangladesh from July 2016 to June 2017. *Klebsiella pneumoniae* were isolated from different clinical specimens from adult hospitalized patients. These isolates were screened for ESBL production according to Clinical and Laboratory Standards Institute (CLSI) guidelines. ESBL production was confirmed by the phenotypic confirmatory double disc synergy test (DDST). **Results:** Among the 500 collected samples 75 *Klebsiella pneumoniae* were isolated. Among them, 68 isolates were selected for confirmatory tests of ESBL according to CLSI guidelines. Finally, 19 isolates were confirmed as ESBL producers by DDST (25.33%). **Conclusion:** In the present study, a large number of isolates are found to be ESBL producers. [Journal of National Institute of Neurosciences Bangladesh, July 2020;6(2): 101-104]

Keywords: Double disc synergy test; extended spectrum β -lactamase; *Klebsiella pneumoniae*; prevalence

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Introduction

Extended spectrum β -lactamases (ESBLs) are a group of β -lactamases with the ability to hydrolyze the extended-spectrum cephalosporin and monobactam antibiotics and they confer in resistance to the penicillin, first, second, and third-generation cephalosporins and monobactams like aztreonam; however, do not affect cephamycins like cefoxitin and cefotetan or carbapenems and inhibited by β -lactam inhibitors such as clavulanic acid, sulbactam, and tazobactam¹⁻³.

ESBLs are of due scientific concern because they are often plasmid-associated and there can be cross-species dissemination of these plasmids. Moreover, these plasmids often carry genes for co-resistance to other antibiotics such as aminoglycosides, fluoroquinolone, tetracycline, chloramphenicol, and sulphamethoxazole-trimethoprim⁴.

Over the last 15 years numerous outbreaks of infection with organisms producing extended spectrum β -lactamases (ESBLs) have been observed worldwide⁵.

Extended-spectrum β -lactamases (ESBLs) production in *Klebsiella pneumoniae* was first found in 1983⁶. The prevalence of ESBL-producing *K. pneumoniae* has greatly increased since that time, and ESBL production has hindered effective management of infection⁷. Till now, *Klebsiella pneumoniae* and *Escherichia coli* remain the major ESBL-producing organisms isolated worldwide⁸. Hence, the present study was attempted to evaluate the extent of prevalence of ESBL-producing strains of *Klebsiella pneumoniae* in Dhaka Medical College hospital (DMCH).

Methodology

This study was carried out at the Department of Microbiology of Dhaka Medical College, Dhaka, Bangladesh from July 2016 to June 2017. This research protocol was approved by the research review committee (RRC) and ethical review committee (ERC) of Dhaka Medical College. Clinical specimens including urine, wound swab, sputum, blood and endotracheal aspirates (ETA) were collected aseptically for bacteriological examination from adult hospitalized patients of Dhaka Medical College Hospital. Written informed consent was taken from all the participants for this study. All samples were inoculated in blood agar and MacConkey agar media, for blood samples primary blood culture was done in Tryptic Soy Broth. After incubation at 37°C aerobically for 24 hours incubated plates were then examined. *Klebsiella pneumoniae* were identified from the lactose fermenting colonies on MacConkey's agar media, if a triple sugar iron (TSI) agar reaction is acidic with gas production but no H₂S, non-motile, indole negative and urease positive in motility-indole-urea (MIU) agar media, citrate utilizer in Simmons citrate agar media. Additional bacterial characteristics including its mucoid colony and oxidase negativity⁹. Clinical and Laboratory Standards Institute (CLSI)¹⁰ has developed screening tests for identifying the ESBL-producing *Klebsiella* species. According to CLSI guidelines, strains showing zone of inhibition of ≤ 22 mm for ceftazidime, ≤ 27 mm for cefotaxime, and ≤ 25 mm for ceftriaxone were selected for conformational tests of ESBL. **Double Disc Synergy Test (DDST)**¹¹: The isolated colonies were inoculated in peptone water at 37°C for 2–6 h. The turbidity was adjusted to 0.5 McFarlands standard and lawn culture was made on Mueller Hinton agar using sterile swab. Augmentin disc (20 μ g of amoxicillin plus 10 μ g of clavulanate) was placed in the centre of plate. Both side of augmentin disc, a disc of cefotaxime (30 μ g) and ceftazidime (30 μ g), were placed with centre to centre distance of 15 mm

to centrally placed disc. The plate was incubated at 37°C overnight. ESBL production was interpreted as the 3rd-generation cephalosporin disc, inhibition was increased towards the augmentin disc or if neither discs were inhibitory alone but bacterial growth was inhibited where the two antibiotics were diffused together. A non-ESBL-producing organism (*Escherichia coli* ATCC 25922) and an ESBL-producing organism (*Klebsiella pneumoniae* ATCC 700603) were used as control strain to assess the performance of the method. Data were analyzed by using Microsoft Office Excel (2013) software (Microsoft, Redmond, WA, USA).

Results

Among the 500 samples collected from DMCH, 75 *Klebsiella pneumoniae* were isolated from different clinical specimens; 14 from urine, 28 from wound swab, 20 from sputum, 7 from ETA and 6 from blood. According to CLSI guidelines for screening among the 75 clinical isolates of *Klebsiella pneumoniae*, 68 were selected for confirmatory tests of ESBL. Double disc synergy test was used to confirm ESBL producing *Klebsiella pneumoniae*.

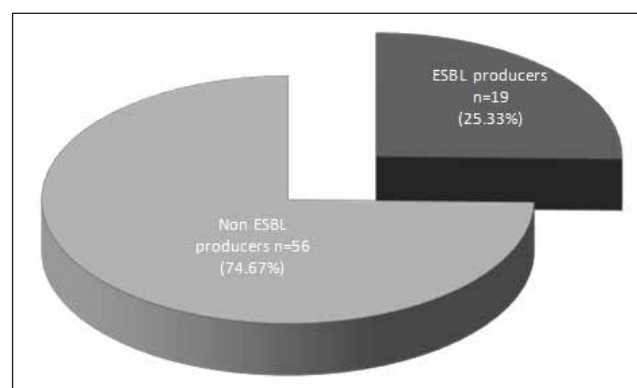


Table 1: Samplewise distribution of ESBL producing *K. pneumoniae*

Sample	Number of <i>K. pneumoniae</i> isolates	ESBL producing number of isolates	<i>K. pneumoniae</i> percentage
Urine	14	4	28.57 %
Wound swab	28	7	25 %
Sputum	20	5	25 %
ETA*	7	2	28.57 %
Blood	6	1	16.67 %

* ETA= Endotracheal aspirates.

The results (Figure 1) showing that 19 isolates were positive by DDST (25.33%). Sample wise distribution of ESBL producing *Klebsiella pneumoniae* is shown in table 1. The highest percentage of ESBL was reported from urine and ETA followed by wound swab, sputum and blood.

Discussion

ESBL producing *Klebsiella* pose great challenges for efficient treatment of infections which requires new antibacterial compounds every now and then. This study focused on the present status of ESBL producing *Klebsiella pneumoniae*. In this study, the prevalence of ESBL producing *Klebsiella pneumoniae* was 25.33%. The present result correlate with another studies reporting 21.2% and 25.65% prevalence¹²⁻¹³. A study from India by Sarojamma and Ramakrishna¹⁴ reported that among the hospital isolates 28% *Klebsiella pneumoniae* were ESBL producer whereas another studies reported that the percentage of ESBL-producing organisms ranged from 4.0% to 83.0% in India¹⁵⁻¹⁶.

However, the present result for the occurrences of ESBL among *Klebsiella pneumoniae* isolates in DMCH is significantly lower than previous studies by Satter¹⁷ and Mostofa¹⁸ who found much higher rate (47.62% and 42.30%, respectively). Exact reason of such reduction of ESBL producing *Klebsiella pneumoniae* is not clear. Physicians informed that now a days the use of carbapenem and polymyxin to treat bacterial infections is common in comparison to ampicillin and cephalosporins (Personal communication). This might have some role in decreasing ESBL production. Physicians also informed that they are aware of the emergence of widespread antimicrobial resistance and to prevent this most of them have adopted some infection control measures. Knudsen and Andersen¹⁹ showed adoption of infection control measures play a significant role in reduction of ESBL producing organisms. Finding of this study like reduction of ESBL producing *Klebsiella pneumoniae* may also be explained by this.

A significant number of ESBL-positive cases are recorded from ETA samples with the percentage of 28.57%. This may be because many of the ETA samples are taken from intensive care unit wards. The result of this study also showed high ESBL percentage (28.57%) from urine samples. Babypadmini et al²⁰ reported 40.0% prevalence for ESBL producing *Klebsiella pneumoniae*, in urinary isolates in Coimbatore, India.

Conclusion

This study highlights the prevalence of ESBL-producing *Klebsiella pneumoniae* in Dhaka medical college hospital, Bangladesh, having a significant percentage. Further studies need to be carried out from other parts of Bangladesh considering ESBL production rate among clinical *Klebsiella* isolates by the standard detection methods so as to control the spread of these infections and also to institute proper therapeutic strategies.

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