

# Prevalence of Extended Spectrum $\beta$ -Lactamase in *Klebsiella pneumoniae* Isolates in a Teaching Hospital of Zahedan City, Iran

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**Background:** Extended-spectrum  $\beta$ -lactamase (ESBL)-producing *Klebsiella pneumoniae* has become widespread in hospitals and is increasing in community settings. Most of *K. pneumoniae* that harbor these enzymes, display resistance to other unrelated antimicrobial agents and thus, often pose a therapeutic dilemma.

**Objectives:** This study was conducted to determine the prevalence of ESBL-producing *K. pneumoniae* in a major university hospital in Zahedan, Iran.

**Materials and Methods:** The susceptibility of 83 *K. pneumoniae* isolates to 12 antibiotics was assessed using Kirby-Bauer disc diffusion method. For the ESBL phenotypic test, double-disk diffusion (DD) method was used.

**Results:** The highest resistance rates of the isolates were seen against cefixime (82%), cefotaxime (81%), ceftriaxone (73%), ceftazidime (72%), and azithromycin (60%), consecutively. The lowest resistance rates were observed against gentamicin (58%), tetracycline (59%), nalidixic acid (59%), and amikacin (63%), consecutively. ESBLs were found in 65% of *K. pneumoniae* isolates.

**Conclusions:** We found that 65% of *K. pneumoniae* isolates produced ESBL. Therapeutic strategies to control infections should be carefully formulated in teaching hospitals. The high percentage of drug resistance in ESBL-producing *K. pneumoniae* suggests that routine detection of ESBL by reliable laboratory methods is required.

**Keywords:** Antibiotic Resistance; Extended Spectrum Beta Lactamase; Antimicrobial Susceptibility; *Klebsiella pneumoniae*

## 1. Background

*Klebsiella pneumoniae* is an important opportunistic pathogen that frequently causes urinary tract infections; however, respiratory tract infections are more serious (1). It is the most common cause of gram-negative septicemia and nosocomial infections. The worldwide development of multidrug resistant (MDR) strains of *K. pneumoniae* is a growing public health issue and is a serious concern for the medical community (2). Extended-spectrum  $\beta$ -lactamase (ESBL)-producing strains of *K. pneumoniae* (ESBLKP) have emerged as a major problem in hospitalized as well as community-based patients (3). In gram-negative bacteria, production of  $\beta$ -lactamases is a major means of resistance to  $\beta$ -lactam antibiotics (4, 5). ESBLs are a group of enzymes that can hydrolyze a variety of  $\beta$ -lactams, including cephalosporins (ceftazidime, cefotaxime, and ceftriaxone), monobactams (aztreonam), and penicillins; however, they do not hydrolyze cephamycins such as cefoxitin. Most ESBLs can also hydrolyze the fourth-generation cephalosporins such as cefepime (6, 7). Until recently, most infections caused by ESBLKP had been mostly described as nosocomial, nursing home-related, or healthcare-associated infections as they typi-

cally infected patients who had been in hospitals or other healthcare facilities (8, 9). Infections with ESBLKP have been associated with higher mortality rates and lower rates of favorable clinical responses to antibiotic regimens. In recent decades, however, these infections have been seen increasingly in patients with no prior contact with the healthcare environment (10, 11).

## 2. Objectives

A better understanding of the dissemination of bacterial resistance to antimicrobial agents is necessary to control the problem. This study focused on the prevalence, susceptibility profile, and evaluation of ESBLKP isolates, obtained from hospitalized patients in Zahedan, Iran.

## 3. Materials and Methods

### 3.1. Specimens

From September 2013 through May 2014, 1580 clinical specimens were collected for bacterial culture from patients attending Khatam Al-Anbiya Hospital, Zahedan

University of Medical Sciences, Zahedan, Iran. These included 422 sputum, 914 urine, 117 pus, 100 blood, and 27 cerebrospinal fluid specimens. All clinical isolates of *K. pneumonia* were identified by conventional biochemical tests. Only one isolate of *K. pneumonia* per patient was collected, to avoid repetition of isolates.

### 3.2. Antimicrobial Susceptibility Testing

Antimicrobial susceptibility of *K. pneumonia* was examined by the Kirby-Bauer agar diffusion method using *K. pneumonia* ATCC 700603 as the control strain. Commercially available antimicrobial disks of amikacin (AMK; 30 µg), azithromycin (AZM; 30 µg), cefixime (CFM; 5 µg), cefotaxime (CTX; 30 µg), ceftazidime (CAZ; 30 µg), ceftriaxone (CRO; 30 µg), ciprofloxacin (CIP; 5 µg), gentamicin (CN; 10 µg), imipenem (IMP; 10 µg), nalidixic acid (NA; 30 µg), tetracycline (TE; 30 µg), and trimethoprim/sulfamethoxazole (SXT; 25 µg) were used on Mueller-Hinton Agar (MHA, Hi-Media, Mumbai, India) to test susceptibility. All of antibiotics were purchased from Padtan-Teb, Iran.

### 3.3. Test for Extended-Spectrum β-Lactamase Production

Screening was done by standard disk diffusion method for ESBL production according to criteria recommended by Clinical and Laboratory Standards Institute (CLSI) guidelines (12). Two discs, namely, ceftazidime (30 µg) and ceftriaxone (30 µg), were used for in vitro sensitivity testing using the Kirby-Bauer disk diffusion method. Zone diameters were read according to CLSI criteria. An inhibition zone of ≤ 22 mm for ceftazidime and ≤ 25 mm for ceftriaxone would indicate a probable ESBL-producing strain and need for phenotypic confirmatory testing.

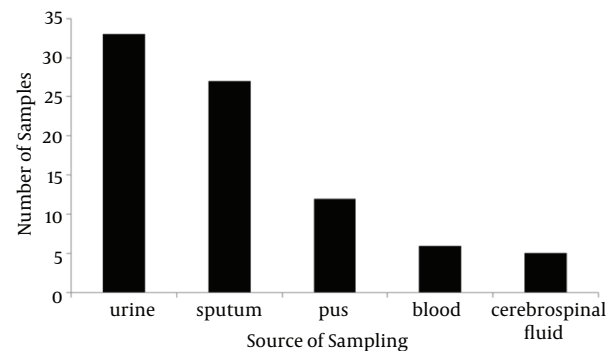
Disk diffusion was used to confirm ESBL production by *K. pneumonia* strains (13). Ceftriaxone (30 µg) versus ceftriaxone/clavulanate (30/10 µg) and cefotaxime (30 µg) versus cefotaxime/clavulanate (30/10 µg) were placed in Mueller Hinton Agar plates, lawned with the test organisms, and incubated at 35°C overnight.

### 3.4. Quality Control

Standard strains of *K. pneumonia* ATCC 700603 and *Escherichia coli* ATCC 35218 were used as internal controls in each susceptibility determination.

## 4. Results

During the study period, 83 strains of *K. pneumonia* were isolated from extraintestinal infections. Urinary tract infections (33 cases, 39.75%) were the most common infections caused by *K. pneumonia*, followed by sputum (27 cases, 32.53%), pus (12 cases, 14.45%), blood (six cases, 7.22%), and cerebrospinal fluid (five cases, 5%) (Figure 1). The analysis of drug resistance patterns showed that the maximum resistance was to cefixime (81.92%) and the least resistance was to imipenem (43.37%). A moderately high resistance was seen to cefixime (82%), cefotaxime (81%), ceftriaxone (73%), ceftazidime (72%), and azithromycin (60%). Moderate resistance was seen to amikacin (63%), azithromycin (60%), tetracycline (59%), nalidixic acid (59%), and gentamicin (58%) (Table 1). About 65% of the 83 strains of *K. pneumonia* were ESBL-positive with the highest frequency being from urine (50%), followed by sputum (17.85%), pus (17.85%), blood (7.14%), and cerebrospinal fluid (7.14%). Of the ESBL positive isolates, 100% was resistant to



**Figure 1.** Distribution of *Klebsiella pneumoniae* Isolates Collected From Patients Based on Source of Sampling

**Table 1.** Antibiotic Resistance of *Klebsiella pneumoniae* Against Twelve Studied Antibiotics<sup>a</sup>

Antibiotics	Resistant (R)	Intermediate (I)	Sensitive (S)
Amikacin	52 (63)	28 (34)	3 (4)
Azithromycin	50 (60)	18 (22)	15 (18)
Cefixime	68 (82)	3 (4)	12 (14)
Cefotaxime	67 (81)	12 (14)	4 (5)
Ceftazidime	60 (72)	13 (13)	10 (12)
Ceftriaxone	61 (73)	14 (17)	8 (10)
Ciprofloxacin	44 (53)	22 (27)	17 (20)
Trimethoprim/sulfamethoxazole	54 (65)	8 (10)	21 (25)
Gentamicin	48 (58)	5 (6)	30 (36)
Imipenem	36 (43)	4 (5)	43 (52)
Nalidixic acid	49 (59)	31 (37)	3 (4)
Tetracycline	49 (59)	27 (33)	7 (8)

<sup>a</sup> Data are presented as No. (%).

**Table 2.** Antibiotic Resistance Pattern of Extended-Spectrum  $\beta$ -Lactamase Producing *Klebsiella pneumoniae* Isolates

Antibiotics	Extended-Spectrum $\beta$ -Lactamase Producing <i>Klebsiella pneumoniae</i>		
	Resistant (R) (%)	Intermediate (I) (%)	Sensitive (S) (%)
Amikacin	61	39	-
Azithromycin	64	18	18
Cefixime	93	-	7
Cefotaxime	100	-	-
Ceftazidime	89	4	7
Ceftriaxone	93	7	-
Ciprofloxacin	57	29	14
Trimethoprim/ sulfamethoxazole	82	7	11
Gentamicin	75	-	25
Imipenem	54	11	36
Nalidixic acid	61	36	4
Tetracycline	79	7	14

cefotaxime, 92.85% to cefixime and ceftriaxone, 82.14% to trimethoprim/sulfamethoxazole, and 78.57% to tetracycline (Table 2). Imipenem was the most effective antimicrobial agents against ESBLK isolates (56.63%). A high MDR, defined as resistant to  $\geq 3$  different classes of antimicrobial agents, was observed among the ESBLK (96.42%).

## 5. Discussion

During the past decade, ESBL-producing Gram-negative bacilli, especially *K. pneumoniae*, have emerged as serious causative pathogens in nosocomial and community-acquired infections worldwide (14). ESBL production rate by Enterobacteriaceae has increased noticeably in two recent decades (15, 16). There are different reports from all over the world regarding the prevalence of ESBLK (16-18). In this study, the prevalence of ESBLK isolates in patients was 65%. In a study performed in Japan, prevalence of 40% (19) and in another one in Berkley, the United States, prevalence of 44% were reported (20, 21). In a study in France, the incidence of this phenotype has been reported to be 30% to 40% in hospitalized and 6% in ambulatory patients (22). Although the prevalence of ESBL has been reported to be 20% in some studies in Southeast Asia, it has been reported to be more than 60% in some regions (23). The occurrence of ESBL among clinical isolates varies greatly in different geographic parts of Iran and is rapidly changing over time. A study in 2010 shows a prevalence of 32% in Kashan (24), 77% in Tehran (25), and 19.02% in Sanandaj (26). In the present study, high resistance of *K. pneumoniae* to numerous antibiotics was observed (Table 1). These results are similar to the reported results from Colombia and Iran (25, 27), showing more than 97% resistance to ampicillin in *K. pneumoniae* isolates. The situation indicates a threat to the community and a possibility that the *K.*

*pneumoniae* could have become resistance to many more antibiotics to which it showed susceptibility earlier. The choice of antibiotics in bacterial infections depends on the local resistance pattern. The results obtained from this study showed a high resistance of *K. pneumoniae* isolates to cefixime, cefotaxime, ceftriaxone, ceftazidime, and trimethoprim/sulfamethoxazole. This is similar to recent articles in which resistances ranged between 50% and 99% for cefotaxime (3, 28) and was up to 20% for trimethoprim-sulfamethoxazole (29). In our hospital, the high resistance of *K. pneumoniae* to cefixime, cefotaxime, ceftriaxone, and ceftazidime was a disturbing finding since these are among the most widely used empirical therapies for febrile infections such as UTI. In the present study, all of the *K. pneumoniae* isolates had broad-spectrum resistance. Messai et al. showed the same figures (30). The MDR-ESBL was seen in 96.42% of our isolates. This was in agreement with findings by Hyle et al. (31) that showed increased annual prevalence of MDR among Extended spectrum beta-lactamase producing *E. coli* and *K. pneumoniae* (ESBL- EK) isolates from 12.5% to as high as 26.9% during a five-year study. According to Moland et al. (32), clinical laboratories must be able to detect important  $\beta$ -lactamases to ensure optimal patient care and infection control. Our study showed the emergence of MDR-ESBLK in our province. Treatment of MDR will become more complex in the coming years because of further limitation of available drugs. Determination of resistant patterns can help us to choose the best antibiotics in such a situation.

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## Authors' Contributions

Study design, data collection, and data interpretation: Ahmad Rashki, Mahbobeh Barakzahi, and Bahman Hormozi; study design, data collection, data interpretation, funds collection, literature review, and manuscript preparation: Ahmad Rashki; study design, manuscript preparation, data interpretation: Zahra Rashki Ghalehnoo; and study concept and design: Ahmad Rashki and Zahra Rashki Ghalehnoo.

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