

Antimicrobial Resistance Patterns in Extended-spectrum β -lactamase Producing *Klebsiella pneumoniae* Isolates in a Razi Hospital Marand, Iran

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Abstract

The prevalence of antibiotic resistance among extended-spectrum β -lactamase (ESBL)-producing *Klebsiella pneumoniae* has increased markedly in recent years. This could be attributed to association of multi drug resistance in ESBL producing isolates. The present study was aimed to determine the antimicrobial resistance profile of ESBL producing *Klebsiella pneumoniae* isolates from various clinical samples. In this study, 250 cases of *Klebsiella pneumoniae* within 9 months from patients referring to Razi Hospital Marand were collected and identified by biochemical tests. ESBL screening and confirmation along with antimicrobial resistance test was done according to the Clinical Laboratory Standards Institute (CLSI) guidelines. Of the 250 isolates, 150 (60%) were identified as having ESBL phenotype. Over 100% of ESBL isolates showed resistance to Ceftazidime and least resistance was found to Meropenem (3.3%). The prevalence of ESBL is increasing day by day in nearly every center of different countries and necessary steps to prevent the spread and emergence of resistance should be taken.

Keywords: *Klebsiella pneumoniae*, Extended Spectrum Beta Lactamase, Antimicrobial resistance..

1. Introduction

Certainly, modern medicine is indebted to developments in antibiotics research areas and antibiotics, by treating and elimination of infectious agents play a critical role in survival and increased quality of life [1]. The precise definition of the term Extended-Spectrum β -Lactamase (ESBL) remains unclear but is generally used to refer to any β -lactamase, 'generally acquired rather than inherent to a species, that is either able to confer resistance to oxyimino-cephalosporins (but not carbapenems), or that has an increased ability to do so, as compared with classic members of its genetic family [2]. ESBL producing strains of Enterobacteriaceae have emerged as a major problem in hospitalized

as well as community based patients [3]. These organisms are responsible for a variety of infections like urinary tract infection (UTI), septicemia, hospital acquired pneumonia, intra-abdominal abscess, brain abscess and device related infections. Organisms producing ESBLs are clinically relevant and remain an important cause of failure of therapy with cephalosporins [4]. ESBLs are primarily produced by the Enterobacteriaceae family, in particular *Klebsiella pneumoniae* and *Escherichia coli* [5]. Bacteria harboring ESBLs may also acquire and most often exhibit additional resistances to other antimicrobial classes such as the quinolones, tetracyclines, cotrimoxazole, trimethoprim, and aminoglycosides, which further limits therapeutic options and thus pose a therapeutic dilemma [6-9]. ESBL - producing *K. pneumoniae* strains have been implicated in numerous outbreaks of nosocomial infections over the last two decades [10,11]. Considering the extensive use of β -lactam antibiotics including third - generation cephalosporins for the treatment of both hospital - and community - acquired infections in Iran, prevalence of ESBL positive clinical isolates is very probable. A single report on these resistant bacteria from Iran denoted the occurrence of *K. pneumoniae* producing ESBL as high as 44.5% [12]. In this study, we determined the prevalence of ESBL production among *K. pneumoniae* isolated from inpatients, their susceptibility patterns, as well as the risk factors associated with the acquisition of these isolates. Today, there is a concern about the spread of such bacteria from hospital to community [13]. Current knowledge of prevalence of ESBL production by commonly isolated organism such as *K. pneumoniae* is necessary to understand the disease burden and to take necessary action to prevent the spread. Therefore the present study was conducted with an objective to find out the prevalence of ESBL producing *K. pneumoniae* and its antimicrobial resistance profile to formulate effective antibiotic strategy and plan a proper hospital infection control strategy to prevent the spread of these strains.

2. Methods

This descriptive cross sectional study, for a period 9 months (from December to August of 2013) on 250 strains of *K. pneumoniae* isolated from clinical samples (blood and urine) of Razi Hospital Marand was isolates, and after test biochemical, All these isolates were subjected to antibiotic susceptibility testing and studied for extended-spectrum β -lactamase production according to the National Committee for Clinical Laboratory Standards Institute (CLSI). The results were interpreted according to the current guidelines of the CLSI [14]. The following antibiotics were tested: amikacin (30 μ g), cefixime (5 μ g), cefotaxime (30 μ g), ceftazidime (30 μ g), cefuroxime (30 μ g), ciprofloxacin (5 μ g), co-amoxiclav (20/10 μ g), co-trimoxazole (1.25/23.75 μ g), gentamicin (10 μ g), meropenem (10 μ g), nalidixic acid (30 μ g), itrofurantoin (300 μ g), norfloxacin (10 μ g) and piperacillin/tazobactam (100/10 μ g) were placed on the Mueller-Hinton agar plates and incubated at 37°C overnight. After overnight incubation, the diameter of each zone of inhibition was measured in mm.

The screening test in order to identify bacteria producing ESBLs in accordance with the instructions NCLS, using the disc antibiotics ceftazidime (CAZ 30 μ g) and cefotaxime (CTX 30 μ g) in combination with clavulanic acid (CLA 10 μ g) (CinnaGen, Iran) was conducted. The standard strain, *Klebsiella pneumoniae* ATCC 700603 as a positive control and *Escherichia coli* ATCC 25922 was used as a negative control [15]. The collected data were statistically analyzed using SPSS version 18 Svrt. $p < 0/05$ was considered statistically significant.

3. Results and Discussion

In this study antibiotic resistance patterns 250 *K. pneumoniae* strains isolated from patients referred to Razi Hospital Marand city, compared to 10 antibiotics were determined using DAD (Disk Agar Difusion). Samples were collected from 139 (55.6%) females and 111 (44.4%) males. Of the 250 *K. pneumoniae* isolates, 150 (60%) ESBL *K. pneumoniae* isolated and 100(40%) non-ESBL producing *K. pneumoniae* isolated. The result of ESBL and non-ESBL producing *K. pneumoniae* recovered from urine and blood given in Table 1.

Table 1. Results of ESBL producing *K.*

<i>K. pneumoniae</i> isolated N=250	Urine		Blood	
	Urine	Blood	Urine	Blood
ESBL	122(48.8 %)	28(11.2 %)		
Non-ESBL	52(20.8 %)	48(19.2 %)		
Total N	174 (69.6%)	76 (30.4%)		

Note: *pneumoniae* in urine and blood specimens.

The mean age of patients was 65.42 ± 19 years. 58% of ESBL-producing isolates were from females and 42% isolates from males. In 10 of the antibiotic resistance of greater than 50% is observed. This difference is meaningful. High-level resistance was for Ceftazidime (100%) and least resistance was found to Meropenem (3.3%) (Table 2).

Table 2. Antibacterial resistance of ESBL and non-ESBL producing *Klebsiella pneumoniae*.

Antibiotic	<i>Klebsiella pneumoniae</i>	
	ESBL	non-ESBL
Amikacin	80(53.3%)	7(7%)
Cefixime	146(97.3%)	24(24%)
Cefotaxime	148(98.6)	24(24%)
Ceftazidime	150(100%)	10(10%)
Cefuroxime	147(98%)	38(38%)
Ciprofloxacin	123(82%)	11(11%)
Co-amoxiclav	117(78%)	72(72%)
Co-trimoxazole	132(88%)	36(36%)
Gentamicin	112(47.6%)	6(6%)
Meropenem	5(3.3%)	0(0%)
Nalidixic acid	130(86.6%)	24(24%)
Nitrofurantoin	43(28.6%)	27(27%)
Norfloxacin	121(80.6%)	35(35%)
Piperacillin/tazobactam	26(17.3%)	0(0%)

The discovery and development of antibiotics was undoubtedly one of the greatest advances of modern medicine. Unfortunately the emergence of antibiotic resistance bacteria is threatening the effectiveness of many antimicrobial agents. Beta-lactamase is the main defending system of gram-negative bacteria against β -lactam antibiotics. Since the first time β -Lactam antibiotics were used, Beta-lactamases evolved simultaneously and they played a major role in treatment failure [16]. Increased outbreak of antibiotic-resistant microorganisms such as extended spectrum beta-lactamase producing gram-negative bacteria among sensitive patients has resulted in spread of dangerous infections. Resistance and transmission of resistant bacteria occurs because of the pressure of improper use of selected antibiotics. The resistant bacteria are highly prevalent, which is most importantly due to over prescription, prescription of inadequate doses of medication, inappropriate treatment, and wrong diagnosis of infectious bacteria by laboratories followed by inappropriate choice of antibiotics for healing or unreliable antibiograms. In the present study, an attempt was made to understand the prevalence of ESBL producing *Klebsiella pneumoniae*. The emergence and spread of ESBL-producing bacteria appear to be mostly caused by

the the widespread use of broad-spectrum beta-lactam drugs. So that nowadays, we are witnessing the increasing rate of the bacteria in different parts of our Hospital. Prevalence of extended spectrum beta-lactamases in *Klebsiella* sp. and *Escherichia coli* are reported to be 19% and 4%, respectively (SENTRY Antimicrobial Surveillance, 2000) [17,18]. In a study conducted in Seoul hospitals in 1993-98, it was revealed that strains of *Klebsiella pneumoniae* and *Escherichia coli* extracted from blood cultures of children produced ESBL (17.9% and 52.9%, respectively) [19]. Another study in 2003 on urine cultures of patients in ICU wards suggested that 25.6% of strains of *Klebsiella pneumoniae* and *Escherichia coli* produced ESBL [17]. Prevalence of ESBLs is reported to be 6% and 87% in India [18,20,21]. These enzymes are also observed in European countries. For example, its prevalence in French hospitals is 40% [17]. Highest outbreak of ESBL from *Klebsiella pneumoniae* were in Western Pacific region (Latin America) (45.4%), Europe (22.6%), United States (7.6%) and Canada (4.9%) [22].

In the Middle East, where Iran is located, prevalence of ESBL-producing *K. pneumoniae* is as follows: Saudi Arabia (2005) 12.2% [23], Lebanon (2003) 20.0% [24], Egypt (2004) 37.5% [25], Turkey (2004) 50.0% [26], and Jordan (200) 80% [27]. In Pakistan (2005), a study performed in Karachi showed an ESBL prevalence of 36.0% in nosocomial *K. pneumoniae* isolates [28]. A higher rate was reported from India where 80.0% of *K. pneumoniae* isolates in a tertiary care center were labeled as ESBL producers [29]. In the present study the ESBL producing *K. pneumoniae* were 60%, respectively. In studies performed throughout the world, the frequency of ESBL positive *K. pneumoniae* was from 17% to 66.7% [30-37]. For example, in United Arab Emirates (2008), out of 130 samples, 42% of *K. pneumoniae* were ESBL positive [33]. In Turkey (2006), 47% of *K. pneumoniae* were ESBL positive [36]. the percentage of *K. pneumoniae* was similar to our study In Tanzania (2009), 63% of *K. pneumoniae* were ESBL positive and the percentage of *K. pneumoniae* was similar to our study [35]. In Skopje (2009), 24.3% of *K. pneumoniae* were ESBL positive and this percentage was Differently to our study [30]. In India (2007), out of 2655 samples, 26.6% of *K. pneumoniae* were ESBL positive and this percentage was lower than our study which can be due to differences in test method, the type of samples and the study population [37]. ESBL producing *K. pneumoniae* showed higher resistance to ceftazidime (100%), cefotaxime (98.6%) and cefuroxime (98%) while lower resistance was seen with meropenem (3.3%), piperacillin/tazobactam (17.3%), and nitrofurantoin (28.6%). In a study conducted in Saudi Arabia (2005), ESBL producing *K. pneumoniae* showed higher resistance to ceftriaxone while lower resistance was seen with

meropenem (4.2%), amikacin (6.3%) and imipenem (8.3%) [38]. Kadar and Angamathu (2005) reported 11% resistance of meropenem which is more than seen in the present study [39]. One of the most striking findings of the present study was the high prevalence of resistant strains among ESBL-producing *K. pneumoniae* to piperacillin/tazobactam. Another study group from Iran reported 33% resistance to this antibiotic combination, which was Much more than our finding [12].

4. Conclusions

Finally, the present study showed high prevalence of ESBL-producing *K. pneumoniae* in our region . Determination of resistant patterns can help us to choose the best antibiotics in such a situation. Also there is a need to emphasize on the rational use of antimicrobials to decrease the spread of ESBL producing bacteria.

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