

MULTIPLE ANTIBIOTIC RESISTANCE AND ESBL PRODUCING *KLEBSIELLA PNEUMONIAE* ISOLATED FROM CLINICAL URINE SAMPLES

H. RAJESHWARI, S. NAGAVENI, AJAY OLI AND R. KELMANI CHANDRAKANTH*

Department of Biotechnology, Gulbarga University, Gulbarga - 585 106

E-mail: ckelmani@gmail.com

KEY WORDS

Antibiotic resistance
Klebsiella pneumoniae
ESBL
Double disc synergy
test

Received on :

23.12.2009

Accepted on :

21.02.2010

*Corresponding
author

ABSTRACT

Extended spectrum β -lactamases (ESBLs) continue to be a major problem in clinical setups world over, conferring resistance to the expanded spectrum cephalosporins. An attempt was made to study ESBL production among *Klebsiella pneumoniae* and to evaluate the antimicrobial resistant pattern in *Klebsiella pneumoniae* isolated from urine sample of the hospitalized patients especially suffering from urinary tract infection. Antimicrobial susceptibility test revealed Ceftazidime, Cefuroxime, Ceftriaxone, Cephalexin and Nalidixic acid showed 94% of resistance, Ampicillin 91%, Gentamicin 88% and Imipenem showed the lowest percentage of resistance (4%). Standard Double disc synergy test (SDD) method showed 90% of the isolates were ESBL producers. Thus, in the present study, a large number of isolates were found to be MDR and ESBL producers. SDD were found to be better method for ESBLs. Continued monitoring of drug resistance is necessary in clinical settings for proper disease management and also an alternative therapy.

INTRODUCTION

Klebsiella Pneumoniae is a non-motile gram-negative bacterium that belongs to the family Enterobacteriaceae and that has become a well-recognized cause of nosocomial infections. Multi-drug resistance *Klebsiella* has been recognized as a cause of hospital acquired infections world wide. (Nima *et al.*, 2008; Keynan and Rubinstein, 2007). They cause different kinds of infections, for example, pneumoniae urinary tract infection and bacteremia in immunocompromised hosts. They are resistant to numerous antibiotics, including extended spectrum Cephalosporins and are becoming an increasing problem in many hospitals, having a significant impact on clinical practice and overall treatment costs, resulting, complicate antibiotic therapy and interfere with empirical therapy (Podschun and Ullmann, 1998).

Antimicrobial resistance among enteric Gram negative bacteria is fast becoming a global public health concern with rapid increase in multidrug resistant organisms (Stephen *et al.*, 2009). *Klebsiella pneumoniae* is a common cause of urinary tract infections, neonatal sepsis and post surgical infections in hospitalized patients. Resistance of enterobacteriaceae to broad spectrum β -lactam antibiotics via ESBL production is an increasing problem worldwide.

Antimicrobial agents are the most important tool available for managing infectious diseases of bacterial origin. Some of ESBL are untreatable; an observation that reflects the formidable challenge that resistance producing strains can pose in terms of disease control and prevention (NNIS, 2003). The prevention of nosocomial infections and their transmission

requires reliable microbiological diagnosis, rational antibiotic prescribing and effective infection control. The most important determinants in treating patients with infections in the ICU is prompt initiation of effective empirical antimicrobial therapy, taking note of the observation that inappropriate empirical therapy affects patient mortality rates (Luzzaro *et al.*, 2006).

Thus in the present study we have isolated *Klebsiella pneumoniae* from urine sample from hospitalized patients. The antimicrobial susceptibility test was carried out to know the rate of resistance of these isolates. And the double disc synergy test was done to check the ESBL producing strain and there synergic effect.

MATERIALS AND METHODS

Bacterial samples

The *Klebsiella Pneumoniae* isolates used in the present investigation were isolated from patients suffering from urinary tract infections from various hospitals of Gulbarga. Clean-catch midstream urine samples were collected in screw-capped bottles and brought to the laboratory for further processing.

Antibacterial susceptibility test

The antibiogram patterns were determined as per the guidelines established by the National Committee for Clinical Laboratory Standards (NCCLS) by the Disk diffusion method on Muller-Hinton agar. The following antibiotics were used: Amikacin (30 mcg), Amoxicillin (30 mcg), Ampicillin (10 mcg), Tetracycline (30 mcg), Ciprofloxacin (5 mcg), Ceftazidime (30 mcg), Cefuroxime (30 mcg), Ceftriaxone (30 mcg),

Cephotaxime (30 mcg), Gentamicin (10 mcg), Nalidixic acid (30 mcg), Co-trimoxazole (30 mcg), Cefotaxime (30 mcg), Cefixime (5 mcg) and Imipenem (10 mcg).

Standard double disc synergy test (SDDS)

Double disc synergy test was performed for the strains pre-incubated in Brain Heart Infusion broth at 37°C and the optimal density of 0.5 with different combination of antibiotic discs on Brain Heart Infusion agar plates as shown Fig 1. The discs were placed at the distance of 30 mm each and incubated overnight. The organisms were considered ESBL producers if the zone of inhibition around the CAZ/CFT showed a clear cut increase towards the Amoxicillin-Clavulanic acid.

RESULTS AND DISCUSSION

Klebsiella pneumoniae is an opportunistic pathogen and is a causative agent of several kinds of infections in humans. It is one of the major pathogen in nurseries, communities and hospitals, intensive care units in spite of many effective antibiotics now available. (Eisen *et al.*, 1995)

In the present study 50 *K. pneumoniae* strains were isolated from clinical urine samples in hospitalized patients suffering from urinary tract infection. With a period of 2 months (June and July 2007) in Gulbarga.

Since the introduction of beta-lactam antibiotics in the clinical settings, these antibiotics have been mostly in injudicious use for therapeutic purpose. Their widespread use has led to the emergence of various enzymes that are capable of hydrolyzing and lactamases. Initially, the resistance was observed only to penicillins and early cephalosporins. Later, an increase use of broad-spectrum cephalosporins has led to the mutation of genes encoding these enzymes and emergence of ESBLs. The first ESBL was discovered in Western Europe in the mid-1980s, and subsequently in the USA in late 1980. The resistant organisms are now a worldwide problem. They can be found in a variety of Enterobacteriaceae, however majority of ESBL producing strains are *K. pneumoniae*, *K. oxytoca* and *E. coli*. (Ritu *et al.*, 2006)

Based on the results of drug susceptibility testing, 8 different antibiotypes 1 consisted of 20 isolates that showed resistance to all investigated antibiotics. It was the most prevalent antibiotype among the isolates (40%). Six isolates were sensitive to all tested antibiotics (antibiotype 2). The remaining antibiotypes consisted of Ampicillin (91%) and Gentamicin (88%). Ceftazidime, Cefuroxime, Ceftriaxone, Cephotaxime and Nalidixic acid showed the highest rate of resistance (94%) and Imipenem lowest resistance (4%) was demonstrated as shown in Table 1.

The use of broad spectrum antibiotics in hospital environments exerts selected pressure on bacteria, results in promoting infections by multi-antibiotic resistance isolates. Present finding showed that the most useful antibiotics such as Ampicillin, Gentamicin, and Cephalosporins showed increase in comparison with previous studies in different countries (Rasool *et al.*, 2003).

Standard double disc synergy test (SDDS) method was used as screening methods for identifying potent ESBL producers with different combination of antibiotic. Ceftazidime and

Table 1: Percentage of antimicrobial susceptibility of *Klebsiella pneumoniae*

Antibiotics	Sensitivity (%)	Intermediate (%)	Resistance (%)
Amikacin	28	22	50
Amoxicillin	8	12	80
Ampicillin	6	3	91
Gentamicin	6	6	88
Tetracycline	6	4	90
Ciprofloxacin	8	10	82
Ceftazidime	6	0	94
Cefuroxime	6	0	94
Ceftriaxone	6	0	94
Cephotaxime	6	4	94
cefixime	6	6	88
Co-trimoxazole	6	3	91
Nalidixic acid	6	0	94
Imipenem	90	6	4

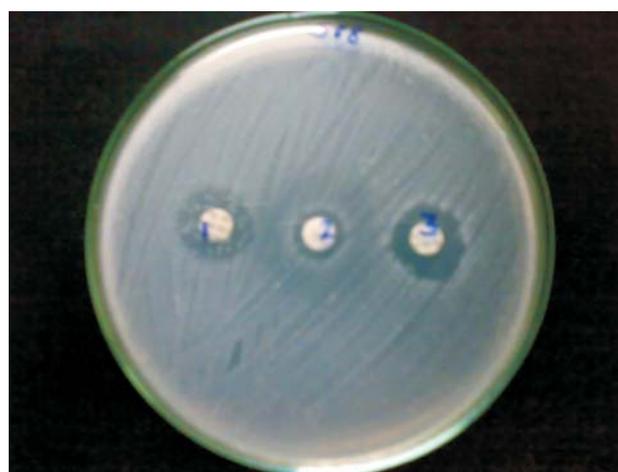


Figure 1: Standard double diffusion of *Klebsiella pneumoniae* Right side the disc of Cefotaxime, left side Amoxicillin-Clavulanic acid and in the middle the Ceftazidime disc

Cefotaxime with Amoxicillin-Clavulanic acid showed 90% of the isolates were ESBL producers.

CONCLUSIONS

Klebsiella pneumoniae is a major pathogen in hospital acquired infection. Emergence of multi drug resistant *K. pneumoniae* is creating a threat to the therapy. And also the production of ESBL from these organisms is one more barrier for curing the infection. So an alternative therapy to fail against these ESBL producing *K. pneumoniae* is required.

REFERENCES

- Eisen, D., Russell, E. G., Roper, E. J., Grayson, M. L. and Turnidge, J. 1995. Random amplified polymorphic DNA and plasmid analysis used in investigation of an outbreak of multiresistant *Klebsiella pneumoniae*. *J. Clin. Microbiol.* **33**: 713-717.
- Keynan, Y. and Rubinstein. 2007. The Changing face of *Klebsiella Pneumoniae* infections in the community. *Int. J. Antimicrob. Agents.* **30**: 385-389.
- Luzzaro F., Mezzatesta M., Mug aioli C., Pirelli M., Stefan S., Amicosante G., Rossellini, G. M. and Toniolo, A. 2006. Trends in production of extended spectrum beta-lactamases among Enterobacteria of medical Interest. Report of the Second Italian Nationwide Survey.

J. Clin. Microbiol. **44(5)**: 1659-64.

NNIS System: National Nosocomial Infections Surveillance (NNIS) System report data summary from January 1992 through June 2003, issued August, 2003. *Am. J. Infect. Control.* **31**: 481-98.

Nima Hosseini Jazani., Mir Davood Omrani., Zahra Sabahi., Masumeh Mosavi and Minoo Zartoshi. 2008. Plasmid profiling of *Klebsiella* sp. and its Relation with Antibiotic resistance at two hospital of Urmia (Iran). *J. Applied Sci.* **8(15)**: 2781-2784.

Podschn, R. and Ullmann, U. 1998. *Klebsiella* spp. as nosocomial pathogens: epidemiology, taxonomy, typing methods, and pathogenicity factors. *Clin. Microbiol. Rev.* **11**: 589-603.

Rasool, S. A., Ahmad, A. and Khan Na A. Wahab, S. 2003. Plasmid borne antibiotics resistance factors among indigenous *Klebsiella*. *Pak. J. Bot.* **35**: 243-248.

Ritu Aggarwal., Uma Chaudhary and Aparna Yadav. 2006. Prevalence and antimicrobial susceptibility of extended spectrum beta lactamases-producing gram-negative bacilli in blood stream infections. *J. Infec. Dis. Antimicrob Agents.* p. 111-114.

Stephen, E., Mshana., Erasmus Kamugisha., Mariam Mirambo., Trinad Chakraborty and Eligius F. Lyamuya. 2009. Prevalence of multiresistant gram-negative organisms in a tertiary hospital in Mwanza, Tanzania. *BMC Research Notes.* **2**: 49.

THE BIOSCAN : SUBSCRIPTION RATES				
		India (Rs.)	SAARC Countries	Other Countries
Individuals	One Year	500	1000(I:C)	US \$100
	Life Member	5000		
Institutions	One Year	1500	3000(I:C)	US \$200
	Life Member	15000		

THE BIOSCAN : MEMBERSHIP FORM

Please enter my subscription for the above journal for the year / life member.

Name:

Address:

E-mail:

Payment Rs. : by DD / MD in favour of
THE BIOSCAN payable at Ranchi, No. Dated is enclosed.

NOTE: FOR MEMBERSHIP THE ABOVE INFORMATION CAN BE SENT ON SEPARATE SHEET