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ORIGINAL ARTICLE

Molecular Characterization of CTX-M Gene Producing *Klebsiella pneumonia*

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ABSTRACT
Several factors govern reason for the increase resistance in the antibiotics. It consist of the prescription without prophylactic measure, sensitivity testing and empirical testing. The subspecies of K. pneumonia is the most prevalent in Pakistan up to 97%. It is difficult and complex to manage the infections caused by these pathogenic organisms. The study of epidemiological and molecular characterization will help in identifying and diagnosis of different types of extended spectrum β-lacatamase (ESBL), the control measurements for such pathogenic strains must be implemented to prevent and reduce their speed. Research was design to detect CTX-M gene incidence and frequency, in ESBL generatingK. pneumonia.
Key words. *Molecular Characterization, Ctx-M Gene, ESBL K. pneumoniae*

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INTRODUCTION

The resistance against antimicrobial agents is continuously emerging among pathogens leading to great risk of treatment failure in case of infectious disease caused by such resistant pathogens. A number of factors are responsible for the increase in antibiotic resistance e.g., prescription of antibiotics without culture and sensitivity testing, empirical therapy, use of antibiotics as prophylactic measure, low dose of antibiotic course for long duration, etc. Such approaches put up selective pressure on bacterial population. As a result susceptible bacteria die while resistant bacteria survive the pressure. If the resistance is plasmid mediated then resistant bacteria are able to spread the plasmids into their surrounding bacterial populations hence transforming bugs into multidrug resistant superbugs[1].
Pathogens have adopted multiple mechanisms to protect themselves against the harmful effect of antimicrobial agents. They can be described as follow:

- Production of enzymes which are capable of deactivation or drug alteration,

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- Modification of the drug site of action so the drug could not be able to bind and act at its respective site of action,
- Changes in the pathways of metabolism,
- Less gathering of the drug in the cell via: driving out of drug actively or by reducing the permeability of drug[2].

One of the chief mechanism in bacterial resistance towards antibiotics is the stimulation of enzymes called ß-lactamase hence providing resistance against ß-lactam group of antibiotics. The reason why this antibiotics group is of great useis that theytheir molecular structures have a mutual four-atom β-lactam ring. They contain derivatives of Penicillin, Cephalosporins, Carbapenemsplus Monobactams [3]. Antibiotics of this group act on bacterial cell wall and prevent manufacturing enzymes of cell wall by binding with them and resulting in catalyzation of peptidoglycan cross linkages. Due to weak cell wall cross linkages, cell lysis occur[4].

Beta-Lactamase enzymes are hydrolytic enzymes which breakdown beta-lactam ring. As a result of modification in structure, the drug deactivates and loses its antibacterial property. These resistance enzymes have been reported to be present in bacteria before the discovery of first ß-lactam antibiotic i.e., Penicillin (Abraham and Chain 1940). After Penicillin, the major discovery of oxyimino-cephalosporins it found to be stable against β-lactamase enzymes. They were named as Extended-spectrum-ß-lactam antibiotics because they were not been able to hydrolyze by the action of beta-lactamase enzyme but resistance soon emerged against them due to the production of new ß-lactamase enzymes classified as "Extended Spectrum ß-Lactamases" (ESBLs). These are actually involve in hydrolysis which hydrolyze β-lactam antibiotics; not only Penicillins but also Cephalosporins and Monobactem[5].

A number of studies have reported presence of high resistance pathogens in Pakistan. One of the resistant pathogen responsible for serious infections reported by different clinical research studies is *Klebsiellaspp*. Among the Klebsiella species, *K. pneumoniae* is the most studied pathogen. It is known to have resistant genes against multiple antibiotics and can lead to prolonged morbidity or even mortality in case of severe infection[6-9].

MATERIAL AND METHODS

In this retrospective study, 39 non-repeat blood culture isolates of *K. pneumoniae* were collected during a period of three months. The samples were collected form the patients admitted in different hospitals of Lahore.. Blood culture was performed using biphasic medium consisting of Brain Heart Infusion (BHI) agar and BHI broth with sodium polyanethol sulphonate as an anticoagulant. *K. pneumoniae* was identified using standard microbiological procedures [10]. Phenotypic evidence of ESBL production was tested by the combination disk method [11**].** *K. pneumoniae* ATCC 700603 and *Escherichia coli* ATCC 25922 were used as controls. Isolates were stabbed into semi-solid nutrient agar butts and were stored at 4°C before retrieval for further investigation.

Gene amplification:

Specifically designed primer were taken for amplification of CTX-M gene that were designed by using Primer 3 Tool.

Table 1: Primers designed for the CTX-M gene

TARGET	PRIMER NAME	SEQUENCE	PRODUCT SIZE
CTX-Beta Lactamase	Forward	TTTTAAAGAATAAGGAATGGG	700
	Reverse	CCCTCCCCGCTAAATACACGCC	700

Polymerase Chain Reaction:

The 10 samples of ESBL producing *K. pneumonia* were amplified by using optimized conditions in programmable thermocycle.

A reaction of 20ul was prepared by using following components of following concentrations.

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Table 2: PCR reaction (master mix) recipe for the amplification of CTX gene

Components	Final Concentration	Volume/Reaction
Mgcl2	25 mM	4ul
PCR buffer (KCL)	10X	3ul
DNTPs	2.5 mM	2.5ul
Forward primer	1 pmoles	1ul
Reverse primer	1 pmoles	1ul
Injection water		11
Taq Polymerase	5 µl	0.12ul
Template DNA	2X	2.5ul
Total volume		25ul

In the initial denaturation the PCR reaction started at 94°C, 2 minutes inorder tocomplete 36 cycles forty five seconds, temperature of 52°C, 40 seconds and elongation was completed at 72 ^{o}C, 45 seconds (Annealing). Interval for last stepwas 10 minutes at 72°C (Elongation)

PCR Product Gel Electrophoresis:

The amplified CTX-M gene was checked on agarose gel electrophoresis. The 5 µl, 6X loading dye was loaded in 25µl, PCR product. 5ul, 1.5 kb plus DNA ladder was applied to compare the bands. 1X, TAE buffer was used to prepare Gel (1.5% Agarose). PCR products were loaded with 5ul, 1 kb plus DNA ladder, thegel was run at the rate of 25 minutes, 80 volts. UV Tran's illuminator was used inorder to visualize the bands.

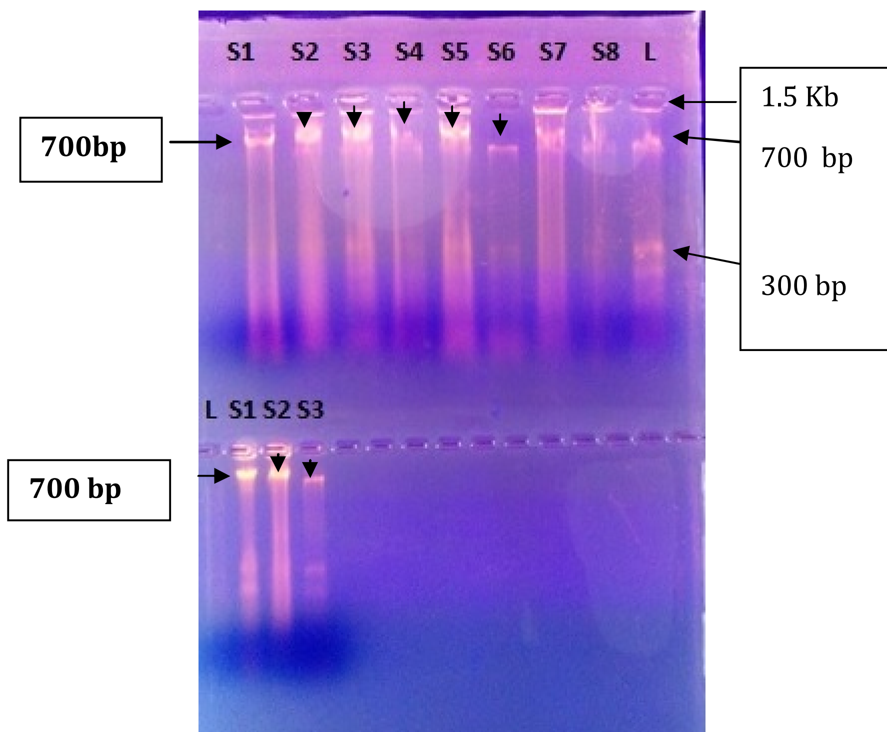

Fig.1. DNA isolation from phenotypic ESBL producing *K. pneumonia.* The amplified product of PCR was run on 1.5% agarose gel. The marking indicate majority of *K. pneumonia* strain isolated showed the CTX-M gene at 700bp marker ladder of 1.5Kb was used.

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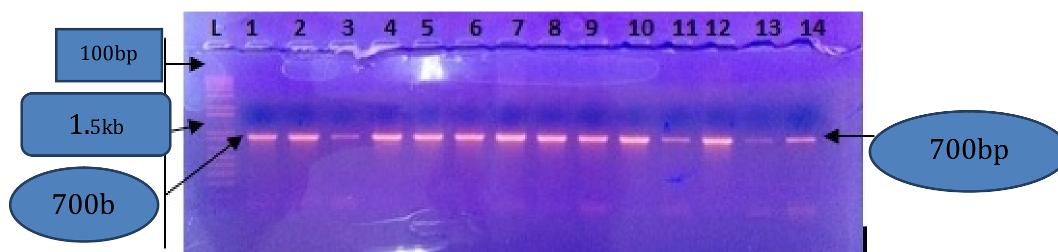

Fig.2. Agarose gel electrophoresis of ESBL producing *K. pneumonia.* The amplified product of PCR was run on 1.5% agrose gel. The marking indicate majority of *K. pneumonia* strain isolated showed the CTX-M gene at 700bp marker ladder of 1.5Kb was used.

DISCUSSION

In present study we also reported the CTX-M gene prevalence in ESBL producing *K. pneumoniae*. CTX-M-type enzymes are a set of class A,ESBLs that are rapidly spreading amongst Enterobacteriaceae worldwide. More than 50 allotypes are known, grouped into six sub-lineages. CTX-M-type ESBLs exhibit powerful activity against cefotaxime and ceftriaxone but generally not against ceftazidime, which has important implications for laboratory detection. Though, quite a lot of CTX-M variants with improved ceftazidimase activity have been sensed. The rapid as well as massive extent of CTX-M-type ESBLs is promptly changing the ESBL epidemiology and, in some geographical areas, these enzymes are currently the supreme dominant ESBLs in *Enterobacteriaceae* [12].

There were very few studies conducted in Pakistan which showed the prevalence of CTX-M gene in *K. pneumonia*. Khan *et al*[13]. Showed a high frequency (96.8%) of CTX-M in ESBL producing *K. pneumonia (*Khan *et al*.). This is a higher prevalence of CTX-M gene as compared to the results of present study.

In a recent study [10] (Hassan, *et al*2014) stated that ESBL *Kleb* frequency as 25.7% while CTX-M positive isolates were 97.4% , in samples collected from a tertiary care hospital in Saudi Arabia.

CONCLUSION

From the current study it may be concluded that in Pakistan the prevalence of CTX-M gene in *K. pneumonia* showed a high frequency (96.8%) of CTX-M in ESBL producing *K. pneumonia*. This is a higher prevalence of CTX-M gene as compared to the results of present study.

REFERENCES

1. Barie PS (2012). Multidrug-resistant organisms and antibiotic management. Surgical Clinics of North America. 92 (2): 345-391.
2. Yeaman MR, Yount NY. (2003).Mechanisms of antimicrobial peptide action and resistance. Pharmacological reviews. 55 (1): 27-55.
3. Holten KB, Onusko EM.(2000). Appropriate prescribing of oral beta-lactam antibiotics. American family physician. 62 (3): 611-620.
4. Fisher JF, Meroueh SO, Mobashery S. (2005). Bacterial resistance to β-lactam antibiotics: compelling opportunism, compelling opportunity. Chemical reviews. 105 (2): 395-424.
5. Bradford PA (2001). Extended-spectrum β-lactamases in the 21st century: characterization, epidemiology, and detection of this important resistance threat. Clinical microbiology reviews. 14 (4): 933-951.
6. Amin A, Ghumro P, Hussain S, Hameed A.(2009). Prevalence of antibiotic resistance among clinical isolates of Klebsiella pneumoniae isolated from a Tertiary Care Hospital in Pakistan. Malaysian Journal of Microbiology. 5 (2): 81-86.
7. Farhat U, Salman AM, Jawad A. (2009). Antimicrobial susceptibility pattern and ESBL prevalence in Klebsiella pneumoniae from urinary tract infections in the North-West of Pakistan. African journal of microbiology research. 3 (11): 676-680.
8. Roshan M, Ikram A, Mirza IA, Malik N, Abbasi SA, Alizai SA.(2011). Susceptibility pattern of extended spectrum ß-lactamase producing isolates in various clinical specimens. J Coll Physicians Surg Pak. 21 (6): 342-346
9. Shafiq M, Rahman H, Qasim M, Ayub N, Hussain S, Khan J, Naeem M.(2013). Prevalence of plasmid-mediated AmpC beta-lactamases in Escherichia coli and Klebsiella pneumonia at tertiary care hospital of Islamabad, Pakistan. Eur J Microbiol Immunol (Bp). 3 (4): 267-271.
10. Forbes BA, Sahm DF, Weissfeld AS.(2002). Bailey and Scott's diagnostic microbiology. 11th ed. St. Louis: Mosby
11. Performance standards for antimicrobial susceptibility testing-9th information supplement: M100-S17. Wayne, PA, USA: NCCLS; 2007. National Committee for Clinical Laboratory Standards
12. Hassan H, Abdalhamid B. (2014). Molecular characterization of extended-spectrum beta-lactamase producing Enterobacteriaceae in a Saudi Arabian tertiary hospital. The Journal of Infection in Developing Countries. 8 (03): 282-288.

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13. Khan E, Schneiders T, Zafar A, Aziz E, Parekh A, Hasan R.(2010). Emergence of CTX-M Group 1-ESBL producing Klebsiella pneumonia from a tertiary care centre in Karachi, Pakistan. The Journal of Infection in Developing Countries. 4 (08): 472-476.

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