Molecular Study on plasmid profiling of clinical isolates of antibiotic resistant Klebsiella from Chittagong city

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Abstract: *Klebsiella* is a common cause of various infections. Plasmids are extra-chromosomal circular deoxyribonucleic acid molecules (DNA) that can exist and replicate independently of the chromosomes in bacteria. In recent year’s Plasmid-mediated multi drug resistance (MDR) in different bacterial species are well recognized. Plasmids can encode genes for antibiotic resistance or virulence factors and also serve as markers of various bacterial strains. This study was done to observe the presence of plasmid in various multidrug resistant clinical isolates of *Klebsiella* from Chittagong city of Bangladesh. To detect the molecular causes of multidrug resistance in *Klebsiella*, ten MDR *Klebsiella* isolates which showed mucoid phenotype on MacConkey media were selected for plasmid-DNA extraction procedure and agarose gel electrophoresis. The present data showed that all the isolates were resistant to multiple antibiotics and among them, 70% *Klebsiella* isolates harboured plasmid. The high occurrence of plasmid in MDR *Klebsiella* isolates indicates that plasmids might have epidemiological significance.

Keywords: *Klebsiella*, Nosocomial infection, Multidrug resistance (MDR)

Introduction

*Klebsiella* species is a Gram-negative, non-motile, encapsulated, lactose fermenting, facultative anaerobe belonging to the Enterobacteriaceae family. *Klebsiella* is the most common causative agent of nosocomial and community acquired infections (Coque et al. 2008). The emergence of nosocomial *Klebsiella pneumoniae* resistant to almost all available antibiotics, including carbapenem, is an increasing problem worldwide (Deris et al. 2012). *Klebsiella* species causes a variety of infections including urinary tract infections, pneumonia, sepsis, wound infections and infections in the intensive care units (Ullah et al. 2009). Various types of antibiotics are used in hospitals for treating these types of diseases but the development of antibiotic resistance by these bacteria is very high. Emergence of multidrug resistant bacteria is associated with four resistant strategies used by bacteria that diminish the effects of antibiotics (Sikarwar et al. 2011). First one is based on enzymatic modification and inactivation of antibiotics, second is restriction of drug targets access, third is alteration of drug target or even complete diminish of the target and last one is based on phenotypic resistance (Henry et al. 2011). In many cases human abuses of different antibiotics might be a major reason for resistance. Antibiotic resistant gene can occur in nonpathogenic bacteria, which can be transferred via lateral gene transfer (Riaz et al., 2011).

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World Health Organization (WHO) announced antibiotic resistance as one of the three major public health threats of this century (Lachmayr et al. 2009). There is a large reservoir of resistant genes, in bacterial genomes and in extra-chromosomal pieces of DNA that encode different mechanisms of drug resistance (Soulsby 2005). Plasmids are extra-chromosomal, autonomous DNA that may encode products that aid in virulence, pathogenicity, and the spread of resistance among a wide spectrum of bacteria (Lina et al., 2007). *Klebsiella* harbors plasmids encoding many virulence or resistance determinants (Jiang et al., 2010). Plasmids may effect bacterial virulence and antibiotic resistance and serve as epidemiological markers (Hinnebush 1993). The high isolation rate of drug resistant bacteria especially *Klebsiella* in patients implicated a great necessity to study the drug resistance pattern of this organism and to determine the factors that are responsible for the development of this character. The aim of the present study was to identify the presence of plasmid and also to find the molecular causes of multidrug resistance in *Klebsiella* species in Chittagong city.

Materials and Method

A total of 99 *Klebsiella* isolates associated with *Klebsiella* infection were isolated from 502...
individual patients. Clinical samples included urine, pus, wound swabs and sputum. Clinical samples were collected from Chevron Diagnostics and Surgiscope Hospital. All isolates were isolated and identified according to the standard microbiological method. The sensitivity tests to *Klebsiella* were carried out with 5 antibiotics such as, Azithromycin, Amoxicillin, Cefexime, Nalidixic acid and Ciprofloxacin on Mueller-hinton agar by modified Kirby-bauer disc diffusion technique. To detect the molecular causes of multidrug resistance in *Klebsiella*, 10 MDR *Klebsiella* isolates which showed mucoid phenotype on MacConkey media were selected for plasmid -DNA extraction procedure and agarose gel electrophoresis.

**Isolation of Plasmid:**

A single bacterial colony was grown in 2ml of LB medium in a test tube at 37°C with vigorous shaking. 1.5ml culture was poured into a microfuge tube and then centrifuged at 12,000g for 30 seconds at 4°C in a micro centrifuge. The supernatant was discarded. The bacterial pellet was re-suspended in 100µl of ice-cold solution I by vigorous vortexing. To the mixture, 200µl of freshly prepared Solution II was added, mixed and the tube was stored on ice. Then to the mixture, 150µl of ice-cold solution III was added mixed and the tube was stored on ice for 3-5 minutes. Then the tube was centrifuged at 12,000g for 5 minutes at 4°C in a micro centrifuge. Then the supernatant was transferred to a fresh tube. To the mixture, an equal volume of phenol: chloroform was added and mixed by vortexing. After centrifuging at 12,000g for 2 minutes at 4°C in a micro centrifuge, the supernatant was transferred to a fresh tube. Then the double-stranded DNA was precipitated with two volumes of ethanol at room temperature. The ethanol was mixed by vortexing and the mixture was allowed to stand at room temperature for 2 minutes. Then the mixture was centrifuged at 12,000g for 5 minutes at 4°C in a microfuge. Then the supernatant was removed by gentle aspiration. Then the tube was allowed to stand in an inverted position on a paper towel to allow all of the fluid to drain away. Any drop of fluid adhering to the walls of the tube was removed. Then the pellet of the double-stranded DNA was rinsed with 1 ml of 70% ethanol at 4°C. The supernatant was removed and the pellet of nucleic acid was allowed to dry in air for 10 minutes and then was stored in the deep freeze.

Plasmid DNA was separated by gel electrophoresis in 1% agarose slab gels in a Tris-borate EDTA (EDTA) buffer at room temperature. A voltage of 1-5 V/cm (measured as the distance between the electrodes) was applied by a power pack. Briefly, the gel was stained with ethydium bromide (0.5gm/ml) for 30-45 minutes. The gel was examined by UV light. (Sambrook *et al.* 1989)

**Result**

Ten multidrug resistant (MDR) *Klebsiella* isolates were examined for the presence of plasmid. Analysis of plasmid DNA by agarose gel electrophoresis revealed that all 7 out of 10 isolates contained plasmid forming a unique banding pattern (Figure.1).

**Figure.1: Plasmid profile of *Klebsiella* isolates.**

**Discussion**

The emergence of multidrug-resistant (MDR) bacteria is inevitable, and has presented a global medical challenge. *Klebsiella* is a well-known cause of community-acquired bacterial pneumonia, but the great majority of infections are hospital-associated (Damian *et al.* 2009). In recent years plasmid-mediated multi drug resistance (MDR) in different bacterial species are well recognized. Wei *et al.*, (2006) identified KPC-2 gene which is located on an approximately 60-Kb plasmid in a carbapenem resistant isolate of *Klebsiella pneumoniae*. Plasmids can encode genes for antibiotic resistance or virulence factors and also serve as markers of various bacterial strains. Acquisition of antibiotic resistant properties of a bacterium could be its inherent properties or occurs due to chromosomal mutation (s) or by acquiring extra-chromosomal DNA plasmid (Mandal *et al.* 2004). Therefore the study of plasmids is very much important to clinical microbiology.

The appearance of plasmid associated with important phenotypic characteristics were found by analyzing of plasmid profiles. To determine, whether the antibiotic resistance was plasmid-mediated or not, ten MDR isolates of *Klebsiella* were subjected to plasmid -DNA extraction procedure and agarose gel electrophoresis. During plasmid DNA extraction from 10 MDR mucoid *Klebsiella* isolates, analysis had shown that 7 isolates tested contained plasmids
The present data showed that all the isolates were resistant to multiple antibiotics and among them, 70% *Klebsiella* isolates harbored plasmid. Nassif *et al.* (1989) reported that *K. pneumoniae* which show mucoid phenotype contains plasmid that encoded a substance is definitely an important virulence factor of *K. pneumoniae*. Most importantly the drug resistance character is most often encoded on plasmids, which can easily be transferred among isolates (Lina *et al.* 2007). The strong associations observed between plasmid profiles and drug resistance patterns suggested that plasmids may have epidemiological significance.

**Conclusion**

Present study suggests that nosocomial infection with MDR *Klebsiella* is a major public health problem in Chittagong, Bangladesh. The high occurrence of plasmid in MDR isolates of *Klebsiella* revealed that resistance to antibiotics could be related to the possession of a higher number of plasmids and plasmids are one of the important ways to spread resistance. The increasing multidrug resistance highlights the need for more refined methods in genetic and epidemiological characterization of *Klebsiella* involved nosocomial infection.

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**References**


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