

# Phenotypic Screening of Aminoglycoside Resistance and their Transferability in Clinical Isolates of *Klebsiella pneumoniae* from India

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## Abstract

**Introduction:** *Klebsiella pneumoniae* is an emerging pathogen associated with multidrug resistance both in hospital and community settings. Aminoglycosides, considered to be second line drug for the treatment of such pathogens, become inactive due to acquisition of various resistance determinants by this organism.

**Objective:** The objective of the study was to screen the aminoglycoside resistant *Klebsiella pneumoniae* from a tertiary referral hospital of northeast India and their transmission dynamics.

**Method:** A total of 177 consecutive, non-duplicate, clinical isolates of *Klebsiella pneumoniae* were collected from patients from a period of September 2013 to February 2014. Screening for aminoglycoside resistance was performed. Transferability of aminoglycoside resistance was done by transformation assay. Genetic stability was checked by consecutive serial passage of 70 days. Incompatibility types were determined by PCR based replicon typing.

**Result:** Among 177 clinical isolates, 94 were screened to be resistant towards aminoglycoside group of antibiotics. The aminoglycoside resistance determinant was found to be transferable when transformants were selected in gentamicin (100 µg/ml) screen agar. Coresistance was also shown by these isolates. Gentamicin resistance was lost after 47 consecutive serial passages. F inc type (n = 17) was more predominant, followed by K/B (n = 11), Y (n = 13), I (n = 9) and P (n = 8) when plasmids were typed by PCR based replicon typing.

**Conclusion:** This study highlighted the transmission dynamics of aminoglycoside resistance determined which pose threat to the treatment option in hospital settings.

**Keywords:** Multidrug resistance; Aminoglycosides; Transferability; *Klebsiella pneumoniae*

## Introduction

*Klebsiella* is known to be the second most important multidrug resistant organism within enterobacteriaceae family after *Escherichia coli*. *Klebsiella pneumoniae* often reported to produce carbapenemase (KPC) leaving the carbapenem antibiotics inactive on them. The organism too often exhibit resistance towards quinolone and aminoglycoside group of antibiotics [1] that continue to play an important role in antimicrobial therapy against both gram negative and gram-positive pathogens, usually in combination with β-lactam agents [2]. Resistance to aminoglycosides can be widespread and has primarily been the result of aminoglycoside inactivation through the chemical processes of acetylation, phosphorylation, and/or adenylation, with varying effects depending upon the particular agent [3,4]. Recently another mechanism i.e, acquired 16S methyl transferase is also known to play a significant role in aminoglycoside resistance. These mechanisms, when combined with additional coexisting multidrug resistant trait, pose severe threat to therapeutic regime. Till now there is a paucity of data regarding aminoglycoside resistance among gram negative pathogen from this geographical part of the world. Thus, this study was undertaken to screen aminoglycoside resistance and their transferability within member of enterobacteriaceae family.

## Methods

### Sample size

A total of 177 consecutive, non- duplicate, clinical isolates of *Klebsiella pneumoniae* were collected from indoor and outdoor patients of Silchar Medical College and Hospital, India for duration of six month from September 2013 to February 2014. Isolates were identified based

on conventional microscopical observation, cultural characteristics and biochemical testing methods.

### Phenotypic screening of multidrug resistant *Klebsiella*

Detection of susceptibility in isolates was performed by Kirby bauer disc diffusion method using following antibiotics ciprofloxacin (10 µg), gentamicin (10 µg), amikacin (30 µg), meropenem (10 µg), cotrimoxazole (25 µg), netilmicin (10 µg) and kanamycin (10 µg) (Hi-Media, Mumbai, India). *E. coli* ATCC 25922 was used as quality control strain. Antibiotic susceptibility testing was performed for both wild type and transformants for aminoglycoside group of antibiotic i.e, gentamicin (10 µg), kanamycin (30 µg), netilmicin (30 µg), and amikacin (30 µg). Results were interpreted according to CLSI guidelines [5].

### Transferability

Isolated plasmids were tested for their ability of horizontal transfer by transformation assay. The recipient strain used was *E. coli* JM107. Transformation was carried out by heat shock method [6].

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Transformants were selected on LB Agar plates containing gentamicin (0.25 µg/ml).

### Analysis of stability of plasmid encoding multidrug resistant phenotype

The experiment was performed to determine the vertical transmission dynamics of resistant genes along with their plasmid in subsequent generations. Plasmid stability of screened multi drug resistant (MDR) isolates as well as their transformants was analyzed by serial passages method for consecutive 75 days at 1:1000 dilutions without antibiotic pressure [7].

### Determination of plasmid incompatibility typing

Plasmid incompatibility was determined for wild type as well as transformants by PCR based replicon typing using 18 pairs of different basic replicon primers, targeting FIA, FIB, FIC, HI1, HI2, I1-Ig, L/M, N, P, W, T, A/C, K, B/O, X, Y, F, and FIIA replicons [8].

### Result

On susceptibility testing, 94 (53.6%) organisms out of 177, all of which were isolated from urine and pus (Table 1) were found to be resistant against one of the aminoglycosides, (gentamicin, netilmicin, amikacin, kanamycin) (Table 2) or more antibiotic. All the isolates showed multidrug resistance (MDR) phenotypes. Aminoglycoside resistance was horizontally transferrable. The plasmid could be selected on *E. coli* JM 107 on Luria Bertani agar containing gentamicin. Most of the transformants showed co-resistance to other representatives of aminoglycoside antibiotics (Table 3).

Transformants retained resistance till 70th passage. Gentamicin resistance was lost after 47th passages.

Incompatibility typing could reveal that plasmid encoding aminoglycoside resistance was carried within diverse type of inc group. Among them F inc type (n = 17) was more predominant,

Type of Clinical specimen	
Urine	94
Pus	83

Table 1: Types of clinical specimen obtained in the study.

Sl.no.	Antibiotic	Number	Percentage
1	Gentamicin	20	18.8
2	Netilmicin	23	21.62
3	Amikacin	25	23.50
4	Kanamycin	26	24.44

Table 2: susceptibility testing against aminoglycoside group of antibiotic.

Screen agar	No. of transformants	Co-harboured antibiotic
Ampicillin (100 µg/ml)	70	NET=2, GEN=3, KAN=4, TGC=3, FEP=3, MER=3

FEP: Cefipime, AMP: Ampicillin, GEN: Gentamicin, KAN: Kanamycin, MER: Meropenem, NET: Netilmicin, TGC: Tigicycline.

Table 3: Screening result of transformants.

Incompatible types	Number
F (FIA, Frep, FIC)	17
K/B	11
P	8
Y	13
I	9

Table 4: Different incompatible types.

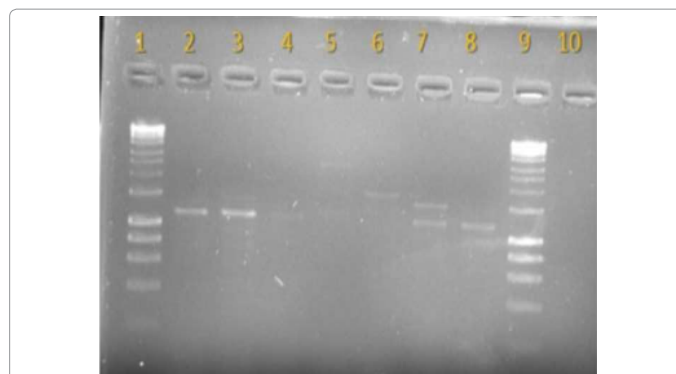


Figure 1: PCR based replicon typing of transformants, Lane 1 and 9 is hyper ladder, lane 2 and 3 showing Inc F, in lane 4 Inc P and in lane 5 and 6 Inc.

followed by K/B (n = 11), Y (n = 13), I (n = 9) and P (n = 8) (Table 4 and Figure 1).

### Discussion

In recent years *K. pneumoniae* is 84 a major organism which is said to be an etiological agent in hospital and community acquired infection [9]. However, increasing resistance to aminoglycosides is becoming a serious clinical problem. Resistance to aminoglycosides is frequently due to the acquisition of modifying enzymes that vary in their substrate ranges, such as acetyltransferases, phosphorylases and adenyltransferases. Aminoglycoside resistant *klebsiella pneumonia* was reported in 2009 [10].

In this study high antibiotic resistance pattern was observed within study isolates family whereas, in this study moderate resistance was observed compared to data of previous studies Jones Goosen, Scwaber. This study underscores the presence and persistence of multidrug resistance within single phenotype, with implications on clinical outcome.

Earlier it was described that plasmid transfer happens due to possible high frequencies present in host, original host carrier, which are not compatible and permits the entry of plasmids. The range of pathogens in which multi-drug resistance or virulence plasmids are stably maintained could thus expand in the future and a better understanding of the evolutionary mechanisms of plasmid host range shifts [11]. In the current study it was observed various multidrug resistance plasmids were highly stable and retained the resistance phenotype till 47 consecutive serial passages. This implies the ability of pathogens to maintain the resistance gene even there is no antibiotic exposure. Thus, therapeutic alternative becomes limited which may lead into treatment failure. Two different studies in India have contrast in their finding where Potron in 2011 has found that *blaKPC-2* encoding plasmid was non typeable while performing PCR based replicon typing [12]. Though later Kumaraswamy in 2011 described that enterobacteriaceae (*Klebsiella* and *E. coli*) were harboring some resistance gene within inc A/C type plasmid [13]. In this study it was observed that this centre, F inc type was more predominant followed by I and Y inc type. Also, it is evident from current investigation F inc type plasmids were encoding multiple resistance gene and could propagate within broad host range. Thus, this inc group could possibly responsible for horizontal transmission of multidrug resistance, their maintenance, persistence within hospital isolates under antibiotic stress.

*Klebsiella pneumoniae* that produced 16S rRNA methylases 113 were reported in 2003 [14]. These enzymes were found to

confer extraordinarily high levels of resistance to clinically useful aminoglycosides, such as amikacin, tobramycin, and gentamicin [15].

## Conclusion

Aminoglycosides are used to treat both gram positive and gram negative bacterial infections. This group of antibiotic is considered to be a second line drug which is prescribed in combination with other groups in clinical settings. Carriage of aminoglycoside resistance and their horizontal transferability in hospital setting demands urgent need to devise proper antibiotic policy and to slow down their expansion from hospital to community environment.

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