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STUDIES ON PLASMID MEDIATED DRUG RESISTANCE IN CLINICAL ISOLATES OF *KLEBSIELLA PNEUMONIAE*

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ABSTRACT:

Pathogenic bacteria in humans and animals resistant to commonly used antibiotics have become a major global healthcare problem in the 21st century. Indiscriminate and inappropriate use of antibiotics in human and animal medication has resulted in the simultaneous development of resistance to several antibiotic classes, creating very dangerous multidrug resistant (MDR) bacterial strains. The widespread emergence of multidrug resistance among bacterial pathogens has become one of the most serious challenges in clinical therapy. The most frequent type of resistance is acquired and transmitted horizontally either through conjugation, transformation or transduction of plasmid. In this regard, the present study was undertaken to investigate the role of plasmid in antimicrobial resistant strains of Klebsiella *pneumoniae,* isolated from the human urine, ear swab and lung aspirates.

KEYWORDS: *Klebsiella pneumonia,* Antibiotic Resistance and Plasmid curing.

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INTRODUCTION

Antimicrobial resistance / drug resistance occurs when microbes survive exposure to antimicrobial agents because of genetic changes in the microbes.

Viruses, fungi and parasites can become resistant, but the greatest problems have occurred with bacteria. Antibiotic resistance in bacteria, which was rare before the dawn of antibiotic era has increased tremendously, mainly because of over-use/misuse of antibiotics and transfer of resistance genes horizontally among bacteria (Nwosu *et al*., 2001; Seveno *et al*., 2002, Poole *et al*, 2001). Many antibiotic resistance genes reside on transmissible plasmids, facilitating their transfer. Exposure to an antibiotic naturally selects for the survival of the organisms with the genes for resistance. In this way, a gene for antibiotic resistance may readily spread through an ecosystem of bacteria. Once a bacterium becomes resistant to an antibiotic, it is unable to return to its previous state of vulnerability. Resistance will be passed on to all of the daughter cells of the resistant microbe. Antibiotic-resistance plasmids frequently contain genes conferring resistance to several different antibiotics. With these concerns, the present study was aimed to inspect the responsibility of plasmid in antimicrobial resistance strain *Klebsaiella pneumoniae*, which was isolated from the clinical isolates of human pathogens.

MATERIALS AND METHODS

Antibiotic sensitivity Test

The clinical samples (urine, ear swab, lungs) from patients referred for diagnosis by physicians were collected from Scudder Lab, Nagercoil. After that it was placed on Nutrient agar plates using serial dilution techniques, and then the plate was kept for incubation at 37°C for 18 – 24 hrs. Morphologically different colonies were selected and then subjected to various biochemical tests (Cappuccino and Sherman, 2006). Antibiotic resistant strain was isolated using antibiotic sensitivitypattern using disc diffusion assay. (Antibiotics which were used for the assay includes Ofloxacin, Gatifloxacin, Meropenem, Ciprofloxacin, Amoxyclav, Norfoxacin and Chloropromazine). The resistant strains were used for further studies

Plasmid Curing

The 24 hrs old cultures of the isolates were grown in sterile nutrient broth containing various concentrations of SDS (0.5%, 1%,

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1.5% and 2%). The tubes were incubated at 37˚C for 24 hrs. After the incubation the isolates were reinoculated in sterile nutrient broth and incubated further for 24 hrs. The cured isolates were checked for their antibiotic sensitivity (Sijhary1984).

Agarose gel electrophoresis for curing plasmid

Plasmid isolation was performed using the alkaline lysis method described by Bimboim and Doly**,** 1979. The plasmid profiling was carried out by sub-culturing those isolates onto nutrient agar plate and incubated for 24 hours at 37˚C. The isolate was harvested into Eppendorf tube. 200 µl of buffer 1A (Cell Resuspension Buffer) was added and vortexed for proper mixing. Two hundred µl of the Cell lysing solution was added. The tubes were Inverted 20 times at room temperature. Add 300 µl of ice-cold buffer 2B (Neutralization solution), vortexed and keep on ice for 30 minutes, centrifuged at 3000 × g for 15 minutes. To supernatant, 700 µl of chloroform was added and centrifuged at 3000 × g for 10 minutes. To 500 µl aqueous layer, 1 ml of absolute ethanol was added and kept on ice for 1 hour. This was centrifuged at 3000 × g for 30 mins and the pellet was washed in 70% ethanol. 100 µl of buffer 3C (Equilibration Buffer) was added to the dried pellet. After the extraction agarose gel electrophoresis was carried out on the plasmid DNA at 90 V for 60 minutes. It was viewed under UV transilluminator for the band harboring plasmid.

RESULT AND DISCUSSION

Ten different colonies were screened from the human samples based on their colony morphology. The colonies having characteristic features such as dirty white, mucoid, pale yellow and dome shaped were selected for further studies. The selected strains were named as U1, U2, U3, ES1, ES2, L1, L2, L3, L4,L5 (Table 1). Among the ten colonies seven were identified as *Klebsiella* sp. three were identified as *Bacillus* (Table 2). Further studies were deal with *Klebsiella sp.* because it is well known to most clinicians as a cause of community acquired bacterial pneumonia. In recent years Klebsiella have become important pathogens in nosocomial infection (Nordmann *et al.*, 2009). In appropriate antimicrobial treatment as well as its overuse has become a contributing factor for the emergence of resistant strains of bacteria. It has become a common practice now-a-days to prescribe the antibiotics for the indications which their use is

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not warrented , even incorrect or sub – optimal antibiotics are frequently prescribed in the treatment of infections that usually can be resolved without treatment.

Table 1. Isolation of microorganism from human samples

S.No.	Source	No of colonies obtained
1	Urine sample	U1, U2, U3 (3)
2	Ear swab	ES1, ES2 (2)
3	Lung sample	L1, L2, L3, L4, L5 (5)

Table 2. Biochemical Test for isolated pathogens

S.No.	Isolates	IMVIC Test						Enzyme Test			Identified Organism
		I	MR	VP	CI	O	CA	Starch	Casine	Urease	
1	U1	-	+	-	-	+	+	+	_	+	*Bacillus sp.,*
2	U2	+	-	+	+	-	+	-	+	+	*Klebsiella pneumonia*
3	U3	+	-	+	+	-	+	-	+	+	*Klebsiella pneumoniae*
4	ES1	-	-	-	-	-	+	+	+	+	*Bacillus sp.,*
5	ES2	+	+	-	+	-	-	+	+	-	*Klebsiella pneumoniae*
6	L1	+	-	+	+	-	+	-	+	-	*Klebsiella pneumoniae*
7	L2	+	-	+	+	+	-	+	+	-	*Klebsiella pneumoniae*
8	L3	-	-	-	-	-	+	+	+	+	*Bacillus sp.,*
9	L4	+	-	+	+	-	+	+	-	-	*Klebsiella pneumoniae*
10	L5	-	+	+	+	-	+	+	+	-	*Klebsiella pneumoniae*

The antibiotic sensitivity result indicates that *Klebsiella pneumonia isolates identified* from urine and lung samples U2 & L1 showed exceedingly resistance to various antibiotics when compared with other 5 *Klebsiella* isolates. Based on this property U2 & L1 were selected for further studies (Table 3).

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Table 3. Antibiotic sensitivity test for *Klebsiella pneumonia*

Strain / Antibiotics	U2	U3	ES2	L1	L2	L4
Meropenem	R	22mm	23mm	26mm	21mm	25mm
Norfloxacin	R	30mm	R	R	29mm	32mm
Gatifloxacin	15mm	32mm	22mm	14mm	33mm	36mm
Chloropromazine	19mm	29mm	31mm	R	32mm	30mm
Ofloxacin	R	28mm	15mm	R	29mm	30mm
Ciprofloxacin	R	31mm	12mm	R	30mm	36mm
Augmentin	R	12mm	R	R	13mm	12mm

Plasmid curing was performed for THE *Klebsiella* isolates U2 and L1with SDS (an anionic detergent that is widely used for the disruption of the cell membrane, at the right concentration). The ability of SDS to dislodge plasmid molecules from their site may also account for the earlier observation that SDS prevents the conjugal transfer of R- Plasmid (Mansi *et al.*, 2000) as replication and subsequent segregation of these plasmids would be impaired owing to the lack of attachment sites. It is also possible for SDS, once it reaches the cytoplasm, to interfere with cellular metabolism through its ability to dissociate proteins into their respective subunits thus rendering some enzymes partially or fully inactive.

After plasmid curing the isolates U2 and L1 were further subjected to antibiotic sensitivity tests .The results revealed that the two organism were now sensitive TO THE antibiotics which CLEARLY indicates that the antibiotic resistant property was present in plasmid DNA (Table 4).

Table 4. Antibiotic sensitivity test for plasmid cured *Klebsiella pneumonia*

Strain / Antibiotics	U2	L1
Meropenem	7mm	26mm
Norfloxacin	12 mm	12mm
Gatifloxacin	15mm	14mm
Chloropromazine	19mm	20 mm
Ofloxacin	9 mm	13 mm
Ciprofloxacin	15mm	26mm
Augmentin	R	R

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REFERENCES

Bimboim,H, Doly J, A Rapid Alkaline Extraction Procedure for Screening Recombinant Plasmid DNA, Nucleic Acids Research, 7 (6) : 1513-1523, (1979).

Cappuccino JG and Sherman N, Microbiology A Laboratory Manual, Pearson Education Inc, London. (2006).

Mansi EM, Karen JA and Craig AI, Isolation and curing of the *Klebsiella pneumoniae* large indigenous plasmid using sodium dodecyl sulfate, *J.Res. Microbiol*., 151: 201-208, (2000).

Murray J, Wilson S, Klein S, Yellin A and Loh E, The clinical response to tigecycline in the treatment of complicated intra-abdorminal infections in hospitalized patients, a phase 2 clinical trial, *Antimicrobial Agents and Chemotherapy*, 3:739, (1991).

Nordmann P, Cuzon G, Naas T . The red thread of *Klebsiella pneumoniae* carbapenemase producing bacteria, Lancet Infect Dis 9 : 228 -236,(2009).

Nwosu VC, Antibiotic resistance with particular reference to soil micro organism ,*Res.Microbiol*., 152:421-430, (2001).

Poole K, Multidrug efflux pumps and antimicrobial resistance in P.aeruginosa and related organism, *J. Mol.Microbial. Biotechnology*, 3:225-264, (2001).

Seveno NA, Kallifidas D, Smalla K, Van Elas JD, Collard JM, Karagaeni AD and Wellington EMH, Occurrence and reservoirs of antibiotics resistance genes in the environment, *Rev.Med. Microbio*l., 13: 15-27, (2002).

SijharyTJ, Bermann ML and Enquist LW, Experiments with Gene Fusions Cold Spring Habor Laboratory Press, New York, (1984).