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STUDIES ON MULTI DRUG RESISTANT *KLEBSIELLA PNEUMONIAE*

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ABSTRACT

The wide spread use of antibiotics in hospitals has led to emergence of multidrug resistant organisms of low virulence like *Klebsiella* causing serious opportunistic infections. The purpose of this study was to know prevalence of antibiotic resistance to *Klebsiella pneumoniae* from clinical isolates from patients admitted to hospital in Barshi town. Mechanism of antibiotic resistance developed by *Klebsiella pneumoniae* was to be detected. A total of 10 strain of *Klebsiella pneumoniae* were selected for the study, 55(53.92%) of which were resistant to at least one of third generation cephalosporins. They were studied for antibiotic resistant and mechanism of antibiotic resistance by VITEK-2 machine. All the clinical isolates were isolated from clinical samples urine, pus, sputum, BAL, Blood, catheter and swabs. Antibiotic resistant of 310 *Klebsiella pneumoniae* cultures found were Wild (14); ESBL positive (288); ACQ PASE (15); ESBL CTX-M like (106); ESBL OXA-30 like (2); Carbapenemase (Metallo or KPC) (75); Resistast Cephalosporinase (AmpC) (49); ACQ Penicillinase (20); Inhibitor resistant PASE (IRT or OXA) (8); SHV1 Hyper production (9); Acq. Cephalosporinase except ACC-1 (36) and Penicillinase (9). Antibiotic resistant studied for antibiotics, Ampicillin, Ticaracilline, Amoxicillin, Piperacillin, Cefazolin, Cefuroxime, Ceftriaxone, Ceftazidime, Cefepime, Aztreonam, Doripenem, Ertapenem, Imipenem, Meropenem, Amikacin, Tobramycin, Nalidixic acid, Ciprofloxacin, Moxifloxacin, Tigecycline, Nitrofurantoin and Colistin was carried out using VITEK-2. Antibiotic resistance of *Klebsiella spp*. were studied by MIC and using HI media antibiotic discs.

KEYWORDS

Klebsiella pneumoniae, Multi drug resistant, VITEK-2 and MIC.

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INTRODUCTON

Antibiotic resistant is a global public health problem. The genus *Klebsiella* is widely distributed in nature multi drug resistant organism are resistant are resistant to one or more therapeutic drugs today's highly problematic multi drug resistant bacteria are *Klebsiella pneumonia* it has limited

number of therapeutic options one of the most resistance mechanism in bacteria is beta lactamase production.

Klebsiella pneumoniae is capsulated, non motile opportunistic pathogenic bacteria it cause many infections. *Klebsiella pneumoniae* is inherently resistant to penicillins, including semi synthetic broad spectrum penicillins and early cephalosporins due to constitutive production of a chromosomally encoded class a group beta lactamase.

MATERIAL AND METHODS

Isolation and identification of *Klebsiella pneumoiae* isolates

Multi drug resistant *Klebsiella species* isolated from clinical samples on MacConkeys agar plate. Morphological and biochemical characterizations were done.

Multi drug resistant *Klebsiella pneumoniae* isolates were isolated from patients with Urinary Tract infections. The present bacterial isolate was identified by microscopic, culture, microscopic and biochemical characterizations tests, 16s rRNA analysis and also by using the Vitek®2 system auto analyzer. Pub Win software also used for identification.

Antibiotic Sensitivity Testing and MIC of Antibiotics

Antibiotic Susceptibility testing was carried out for 13 types of antibiotics were performed by using the Disc Diffusion method on Mueller Hinton agar according to the instructions of the Clinical and Laboratory Standards Institute 2012. Discs of Meropenem (10µg), Cefepime (30µg), Amoxycillin (10µg), Gentamicin (10µg), Nitrofurantoin (300µg), Nalidixic acid (30µg), Tigecycline (15µg), Ampicillin (10µg), Imipenem (10µg), Amikacin (30µg), Ertapenem (10µg), Colistin (10µg) and Piperacillin (100µg) were used.

Any bacterial strain which resist to a minimum at least 3 different classes of antibiotics it is regarded as MDR. This method was performed according to CLSI.

RESULTS AND DISCUSSION

There are 04 *Klebsiella species* isolated from the different clinical samples on MacConkey's agar plate their morphological, cultural and biochemical characterizations was done but LS1 was the potent MDR observed. Of which LS1 and LS2 encountered most frequently.

The clinical and standard isolates of Klebsiella were observed by the battery of biochemical tests. The standard *Klebsiella pneumoniae* cultures displayed positive tests for the production of urease, catalase, citrate utilization and fermentation of sugars like lactose, glucose, sucrose, adonitol, mannitol, esculin and melibiose. The organisms exhibited negative reaction for indole production. There was no H_2S production on the Triple Sugar Iron agar but growth of the organism was seen in potassium cyanide. All the clinical isolates exhibiting colonies similarities to *Klebsiella species* were tested biochemically.

The antibiotic sensitivity testing of all the isolates confirmed *Klebsiella pneumoniae*. This testing was done on the sterile Muller - Hinton agar plates. On the basis of resistance pattern to different antibiotics, the bacterial isolates were categorized into three groups i.e. Resistant (R), Susceptible (S) and Moderately Susceptible (MS).

Cultivation and Identification

The clinical samples were collected aseptically inoculated on the plates of Blood agar and Mac Conkey's agar and incubated at 37^{o}C for 24 hours. The identification was done based on the morphological characteristics of the colonies including size, color, shape, hemolytic nature and pigmentation.

Biochemical Characterizations

The suspected colonies of *Klebsiella species* isolated were further identified through the biochemical tests (Barrow and Felthan, 2003)[1] using the standard procedures.

Antimicrobial Susceptibility test

Susceptibility to the antimicrobial agents was determined by using the Disc Diffusion method (Oqunshe, 2006). The antimicrobial agents used were: Meropenem (10µg), Cefepime (30µg), Amoxycillin (10µg), Gentamicin (10µg), Nitrofurantoin (300µg), Nalidixic acid (30µg),

Tigecycline (15µg), Ampicillin (10µg), Imipenem (10µg), Amikacin (30µg), Ertapenem (10µg), Colistin (10µg) and Piperacillin (100µg).

The inocula were prepared by growing the different Klebsiella strains on the separate sterile agar plates and the colonies from the plates were then transferred by means of a loop into 3ml of normal saline. The surface of sterile Muller-Hinton agar plate was consistently inoculated with the organisms using a sterile cotton swab. The cotton swab was further dipped in to the suspension of organism and pushed against the side of the test tube to take out excess fluid. The wet swab was utilized to inoculate the Muller-Hinton agar by uniformly streaking across the surface. By means of a Disc Dispenser, the antibiotic discs were put on to the surface of the inoculated agar and the plates were incubated at 37ºC overnight. The diameter of zone of growth inhibition observed was measured and evaluated to the chart offered by Clinical and Laboratory Standards Institute (CLSI, 2009)[2].

Nucleotide Sequence Accession Numbers

The data of nucleotide sequence of LS1 and LS2 accounted in the present study have been submitted to the DNA Data bank of Japan (DDBJ) sequence database and assigned accession numbers LC371686 and LC373153 respectively.

It is found from the Figure V that the promising isolate LS1 shows the largest inhibition zone diameter i.e. 25mm to Imipenem and Amikacin followed by Ertapenem which is of 23mm in diameter. After that zone of growth inhibition shown by the LS1 is Nitrofurentoin, Meropenem, Colistin, Gentamicin and Piperacillin are 21mm, 20mm, 17mm, 16mm and 15mm respectively. Whereas no zone of growth inhibition is shown by the LS1 to Cefepime, Amoxycillin, Nalidixic acid, Tigecycline and Ampicillin.

Furthermore, it is also clear from the figure V that the promising isolate LS2 shows the largest zone of growth inhibition to the Imipenem which is of 24mm followed by Cefepime, Nitrofurentoin and Colistin which is of 22mm in diameter. Moreover, the zone of inhibition shown by the LS2 to Ertapenem (20mm), Piperacillin (19mm), Gentamicin (18mm), Meropenem (16mm), Amikacin (16mm) and the least zone of growth inhibition shown by the LS2 to the Ampicillin i.e. of 7.0mm. The LS2 shows no zone of growth inhibition to the remaining antibiotics used in the present study.

Discussion

The constantly increasing crisis of resistance to the different antibiotics has been attributed to the misuse and overuse of the antibiotics which necessitates to be regulated under the stringent guidelines (Ventola, 2015)[3]. The infections owing to the antibiotic-resistant place a lot of weight on the economy of any nation as health care price rise (Golkar *et al*, 2014)[4]. The most severe Gram-negative infections happen in health care settings are most frequently caused by Enterobacteriaceae (typically *Klebsiella pneumoniae*) (Yezli *et al*, 2014[5]; Ventola, 2015[3]). The difficult-to-treator untreatable infections caused by Carbapenem-resistant Enterobacteriaceae (CRE) bacteria are on the increase amongst patients in the medical facilities. In the present research work, we studied the antimicrobial resistance patterns of different Klebsiella isolates from the patients. Exactly 310 clinical samples were collected from the patients suspecting bacterial infection. These samples were screened out and in all total 310 isolates were identified as a positive for *Klebsiella species*.

All the promising clinical isolates of *Klebsiella pneumoniae* were tested for their antimicrobial sensitivity on the sterile Muller Hinton Agar medium. All the isolates were found to be multidrug resistant showing resistance to more than three antibiotics. *Klebsiella pneumoniae* has been associated with different types of infections and one of the most important aspects of Klebsiella is the emergence of multi-drug resistant strains particularly those involved in nosocomial diseases.

In vitro data showed the wide range of beta-lactams, amino glycosides, quinolones and other antibiotics which are very useful for treatment of Klebsiella infections (Weisenberg S A *et al*, 2009[6], Chan Y R *et al*, 2009[7]; Adams-haduch J M *et al*, 2009[8]). All confirmed clinical isolates of *K. pneumoniae* were tested further for antimicrobial sensitivity.

In the studies carried out by Archana Singh Sikarwar and Harsh Vardhan Batra (2011)[9], *K. pneumoniae* strains from clinical cases were found highly susceptible to quinolones and amino glycoside, amikacin and gentamycin.

Table No.1: Antibacterial activity of different antibiotics and there inhibition zone diameter (mm)

S.No	Name of Antibiotics (Different concentration)	*Klebsiella pneumoniae* different species Inhibition zone Diameter (mm)	
		LS1	LS2
1	Meropenem	20	16
	MRP-10	I	R
2	Cefepime	0	22
	CPM-30	R	S
3	Amoxycillin	0	0
	AMX-10	R	R
4	Gentamicin	16	18
	GEN-10	S	S
5	Nitrofurentoin	21	22
	NIT-300	S	S
6	Nalidixic acid	0	0
	NA-30	R	R
7	Tigecycline	0	0
	TAC-15	R	R
8	Ampicillin	0	7
	AMP-10	R	R
9	Imipenem	25	24
	IPM-10	S	S
10	Amikacin	25	16
	AK-30	S	R
11	Ertapenem	23	20
	ETP-10	S	I
12	Colistin	17	22
	CL-10	S	S
13	Piperacillin	15	19
	PI-100	R	I

dentification Information	Analysis Time:	4.75 hours	Status:	Final
Selected Organism	97% Probability Bionumber:	Klebsiella pneumoniae ssp pneumoniae 6627734773564052		
D Analysis Messages				

Biochemical Details

2	APPA	-	3	ADO	+	4	PyrA	+	5	lARL	-	7	dCEL	+	9	BGAL	+		
10	H2S	-	11	BNAG	+	12	AGLTp	-	13	dGLU	+	14	GGT	+	15	OFF	+		
17	BGLU	+	18	dMAL	+	19	dMAN	+	20	dMNE	+	21	BXYL	+	22	BAlap	-		
23	ProA	-	26	LIP	-	27	PLE	+	29	TyrA	+	31	URE	+	32	dSOR	+		
33	SAC	+	34	dTAG	+	35	dTRE	+	36	CIT	+	37	MNT	+	39	5KG	-		
40	ILATk	+	41	AGLU	-	42	SUCT	+	43	NAGA	-	44	AGAL	+	45	PHOS	+		
46	GlyA	-	47	ODC	-	48	LDC	+	53	lHISa	-	56	CMT	-	57	BGUR	-		
58	O129R	+	59	GGAA	-	61	lMLTa	+	62	ELLM	-	64	lLATa	+					

Figure No.1: Identification of *Klebsiella pneumonia* on VITEK-2

Susceptibility Information	Analysis Time: 8.50 hours			Status: Final	
Antimicrobial	MIC	Interpretation	Antimicrobial	MIC	Interpretation
+Amoxicillin		R	Meropenem	8	R
+Ampicillin		R	Amikacin	8	S
Piperacillin/Tazobactam	>= 128	R	Gentamicin	<= 1	S
+Cefotaxime		R	Ciprofloxacin	>= 4	R
Ceftazidime	32	R	Levofloxacin	>= 8	R
Cefepime	>= 64	R	Minocycline	>= 16	R
Aztreonam	32	R	Colistin	2	S
Doripenem	>= 8	R	Trimethoprim/Sulfamethoxazole	>= 320	R
Imipenem	>= 16	R			

+= Deduced drug *= AES modified **= User modified

Figure No.2: Antibiotic Susceptibility test on VITEK-2

Phylogenetic Analysis of LS1 *Klebsiella pneumoniae*

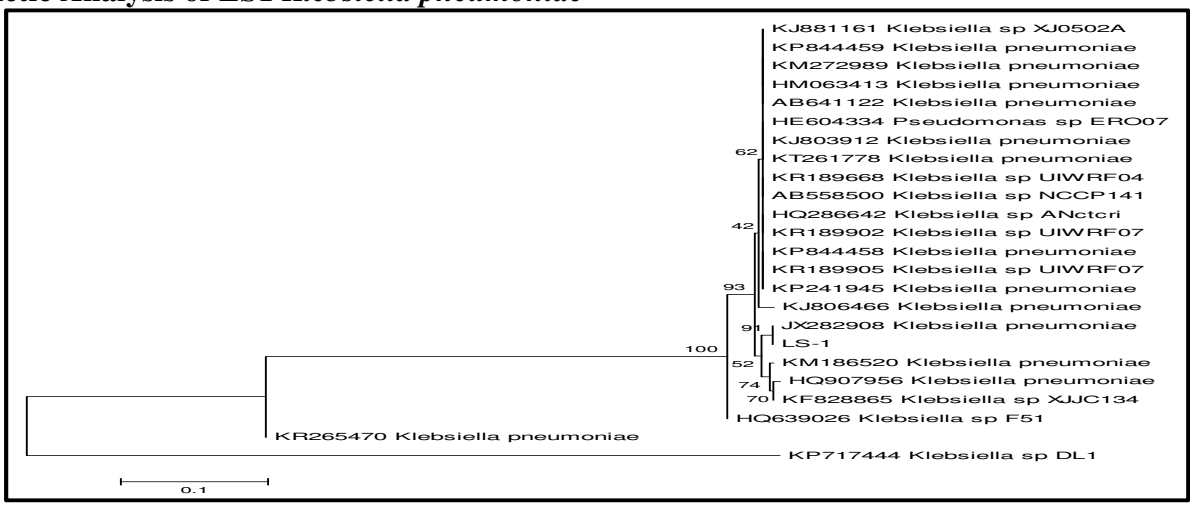


Figure No.3: Phylogenic tree of *Klebsiella pneumoniae LS1*. Phylogenetic analysis of 16s rRNA gene sequence of *Klebsiella pneumoniae LS1*. The percent numbers at the nodes indicate the levels of bootstrap support based on neighbor-joining analyses of 1,000 replicates. The scale bar (0.1) indicates the genetic distance

Phylogenetic Analysis of LS2 *Klebsiella pneumoniae*

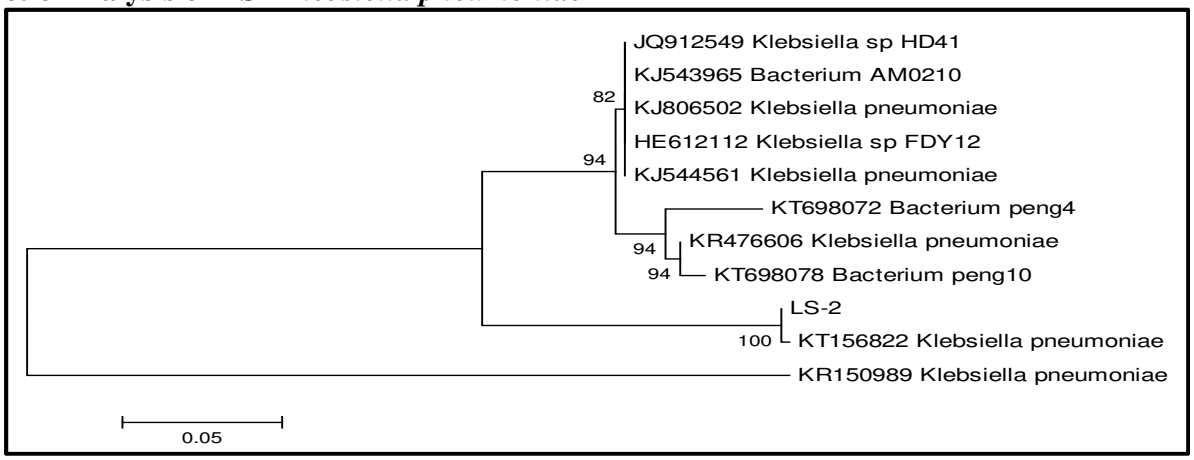
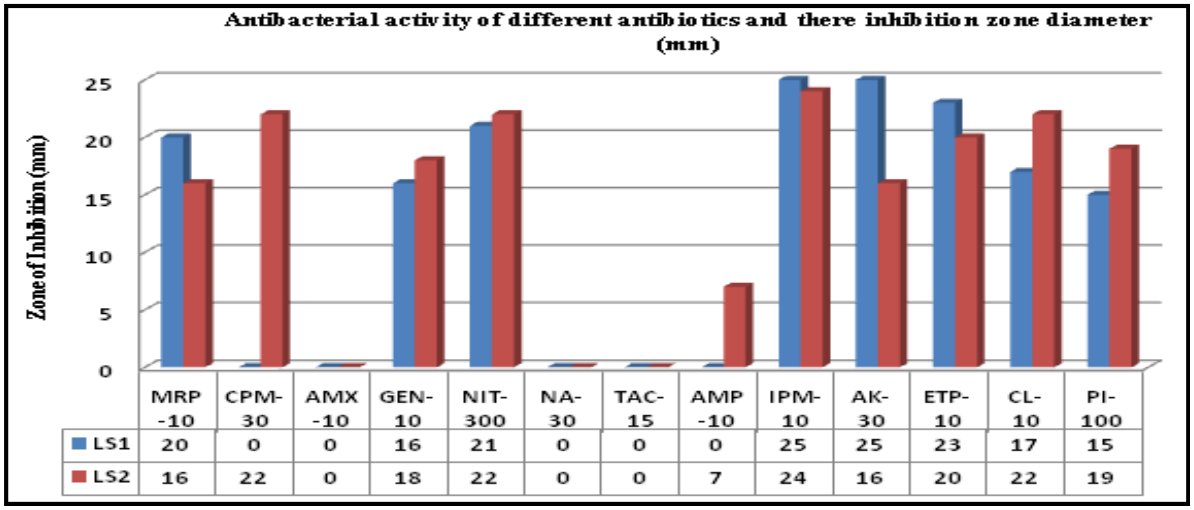


Figure No.4: Phylogenic tree of *Klebsiella pneumoniae LS2*. Phylogenetic analysis of 16s rRNA gene sequence of *Klebsiella pneumoniae LS2*. The percent numbers at the nodes indicate the levels of bootstrap support based on neighbor-joining analyses of 1,000 replicates. The scale bar (0.05) indicates the genetic distance



	MRP -10	CPM-30	AMX -10	GEN-10	NIT-300	NA-30	TAC-15	AMP -10	IPM-10	AK-30	ETP-10	CL-10	PI-100
LS1	20	0	0	16	21	0	0	0	25	25	23	17	15
LS2	16	22	0	18	22	0	0	7	24	16	20	22	19

Figure No.5: Antibacterial activity of different antibiotics and there inhibition zone diameter (mm)

CONCLUSION

Antimicrobial resistance is a global concern not only because it kills but because it increases health costs and threatens patient care (Young Soo S, 2011)[10]. By studying the antibacterial resistance pattern of the pathogens, we can implement and formulate better infection control policies. The developing nationwide, health care guidelines are vital now-a-days due to the increasing resistance patterns. Additionally, by developing the local antibiogram database we can improve the knowledge of antimicrobial resistance patterns in a specific area and which will help in improving treatment strategies. Fulfillment to infection prevention guidelines is crucial for the elimination of major out breaks in the future.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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