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Pathophysiology of Multidrug Resistant *Klebseilla pneumoniae* Causing UTI Infection in Pregnant Women

N. Sreenivasa Rao and Srinivas Munjam*

Department of Microbiology, Kakatiya University, Warangal-506009 (T.S), India.

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Increasing and indiscriminate use of antibiotics for human, veterinary, aquaculture and agriculture along with contamination of water and soil by pharmaceutical industries is the major of increased antimicrobial resistance in recent times. Among the microbes resistant to different drugs, urinary tract infections (UTI) are found to be the highest contributors to antibiotic resistance in microbes. The present study was intended to contribute towards the determination of prevalence of antibiotic resistance of *Klebsiella pneumoniae*, among patients suffering from urinary tract infections who visit hospitals for treatment. Among 2011 samples tested for UTI, 386 (19.34%) samples tested positive. *Klebsiella* accounted for 90 (23.23%) of the positive cases tested, and the isolates were extended-spectrum β-lactamase (ESBL) producers with very high resistance to quinolones, aminoglycosides, carbapenems, and sulfamethoxazole-trimethoprim, but were susceptible to polymixins, Nitrofurantoin, and levofloxacin. The highest prevalence was found among women within the age group of 24-27 years. Of the 90 isolates, 42 isolates were multi drug resistant (MDR) and 48 isolates were non-MDR. Molecular identification using 16S rDNA analysis showed that the isolates were highly resistant organisms and possessed a similarity of more than 99% with UTI isolates reported to have antibiotic resistance.

Keywords: Antimicrobial Resistance; Antibiotics; EMB agar; ESBL Producers; *Klebsiella*, Multi Drug Resistance; 16S rDNA; Urinary Tract Infections.

The advent of antibiotics in the 19th century has transformed medicine and saved millions of lives. However excessive and non-rational use of antibiotics has been the major cause of emerging resistant bacteria and resistance to antibiotics is occurring at a rapid pace, endangering the efficacy of antibiotics[1,2,3]. Lack of new drug development by the pharmaceutical industry due to the challenging regulatory requirements has been a major contributor to increasing antibiotic resistance[4,5,6] Studies have reported that the prominent molecular mechanisms of bacterial resistance to the affectivity of antibiotics are predominantly of four types which include target site modification, destruction or modification of the active moieties of the antibiotic, antibiotic efflux via efflux transporters and decreased membrane permeability leading to reduced antibiotic influx into the cells[7]. Most resistant bacteria possess more than a single mode of resistance mechanisms within the same organism which results in an increased resistance to more than one antibiotics[8, 9].

Klebsiella pneumonia is a multi-drug resistant organism that generally causes urinary

*Corresponding author E-mail: munjam17@gmail.com

tract infections and nosocomial infections. In the case of *Klebsiella,* the organism is naturally unsusceptible to a few classes of antibiotics called as intrinsic resistance. This intrinsic resistance is either attained naturally through any one of the above mechanisms due to the absence of artificially induced antibacterial selection pressure and ampicillin resistance in *Klebsiella* spp[10] is an example. Several studies have shown that *Klebsiella pneumoniae* infections vary from country to country and a study by Ling *et.al*. (2015)[11] reported that the colonization rate among the Chinese population was 66.0%, whereas it was 14.3% among the Maley population, 7.955 among Indians and 11.8% in population of other nations.[12]

Many pathogenic bacteria have developed resistance mechanisms to antimicrobial molecules and can be killed by them. Generally, resistant microorganisms consist of more than one biochemical pathway to overcome resistance to a single class of antimicrobial agent. Usually, a bacterial cell may attain the capability of using more than a single resistance mechanism to obviate the lethal effect of an antibiotic. For instance, resistance to the antibiotic fluoroquinolone, (FQ) usually occurs through three distinct biochemical routes which can be present within the same bacteria and which is generally attained through mutations in genes encoding the target site of the fluoroquinolones which are: a.) DNA gyrase and topoisomerase IV, b) over-expression of efflux pumps that exclude drugs from the cell, and c) protection of the FQ target site by the protein Qnr. Similarly, a few bacterial species have an innate preference for a particular resistance mechanism over others. Among the prominent factors of resistance to antimicrobial agents are bacterial plasmids, which carry several different resistance genes and are capable of conferring resistance to multiple antibacterial agents. The usual mechanism of cross-resistance to multiple antibacterial agents occurs when a resistance mechanism encoded by one gene provides resistance to over one antibacterial compound. Antibacterial-resistant strains and species, colloquially known as "superbugs," are major contributors to the emergence of previously easily controlled diseases. A lot of attention has been given to understand the molecular and biochemical basis of antibiotic resistance in clinical isolates[14,15]. In this work, antibiotic resistance in patients with UTI has been studied and the antibiotic resistance profile of *Klebsiella pneumoniae* causing UTI among pregnant women and the susceptibility to antibiotics was studied.

MATERIALS AND METHODS

Nutrient Agar, Mueller Hinton Agar, Blood Agar, Mac Conkey Agar, Peptone Water, Glucose Broth, Sodium chloride, Beef extract, Yeast extract, Agar agar, Antibiotic discs and test antibiotics were purchased from HiMedia Laboratories, Mumbai, India. All other reagents and chemicals used in the study were sourced from standard suppliers.

The present study was carried out at the Department of Microbiology at Prasanthi Hospital, Hanamkonda, Telangana, India over a period of one year. All 90 isolates of *Klebsiella pneumoniae* that were obtained from various clinical samples and received in the microbiology laboratory from inpatient and outpatient departments of the hospital were included in the study. Several clinical specimens received in the laboratory were processed and, subjected to standard microbiological and biochemical tests followed by molecular identification using 16S rDNA analysis. For the identification of *Klebsiella pneumoniae.* All the identified isolates were further subjected to ESBL screening tests. Among the ESBL producers a highly resistant ESBL producer isolate of *K. Pneumonia* was then selected and further subjected to ESBL phenotypic confirmatory test via the Disc Diffusion method. Antimicrobial susceptibility test for all confirmed isolates was performed according to the Kirby – Bauer disc diffusion method according to the Clinical Laboratory Standard Institute protocols.

Nutrient agar was prepared as per the American Public Health Association method[16]. Blood agar was prepared by mixing 7 ml of sterile defibrinated sheep blood in to 100 ml of melted nutrient agar. MacConkey agar was prepared according to the method of MacConkey (1905) without any modification[17].

Culturing of microbes from urine samples

The sample was inoculated by using a standard inoculation loop (with an internal diameter of 4 mm) on well dried plates of nutrient

agar and MacConkey agar. The inoculated Plates were incubated overnight at 37°C for 24 hours. The number of bacterial colonies that appeared on the plates after the incubation period was counted to determine urine samples with significant bacteriuria. The number of colonies grown after 24 hours and their morphology were noted and recorded. The presence of more than or equal to 10 CFU/ml for a single potential pathogen was interpreted as positive for UTI and the sample was repeated for its accuracy. Those samples having less than 10 CFU/ml were interpreted as negative for UTI.

Antibiotic sensitivity tests: Readymade antibiotic discs were used for the study and the following antibiotics were tested. Amoxicillin, Ceftriaxazone, Cephalexin, Norfloxacin, Levofloxacin, Amikacin, Piperacillin-Tazobactum, Gentamycin, Imepenem, Meropenem, Colistin, Linezolid, Sulfamethaxzole-Trimethoprim, Nitrofurantoin, Vancomycin.

The Kirby-Bauer method recommended by the CLSI guidelines was followed testing of antimicrobial susceptibility of the isolates towards different antibiotics which included antimicrobial disk susceptibility tests were performed for studying isolates[1]. The accuracy and reproducibility of the test were dependent on maintaining a standard set of procedures.

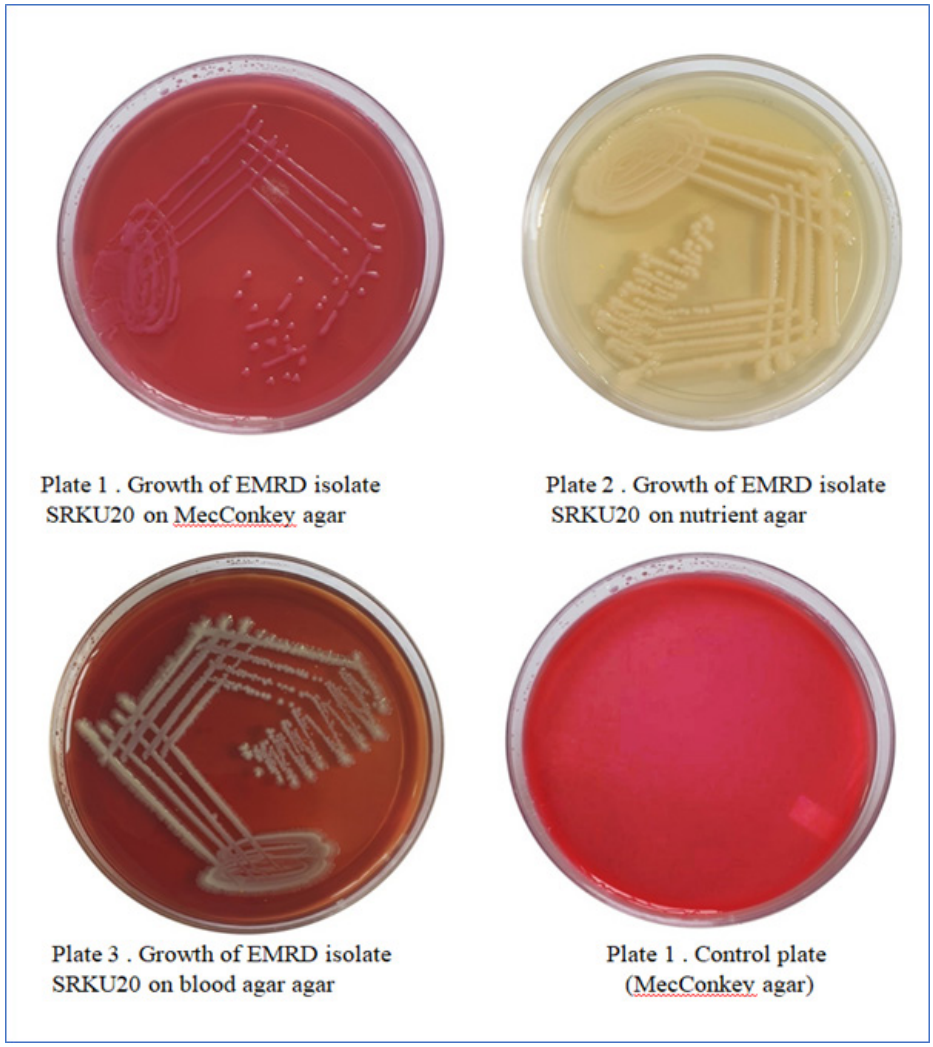

Fig. 1. Cultural characteristics of *Klebsiella pneumoniae* isolate

Characterization of *Klebsiella pneumoniae* isolates

The bacterium has been obtained as pure culture from the urine sample was identified based on morphological, cultural, microscopic and biochemical characteristics. Cultured petriplates were observed under high intensity of light for colour, shape, size, appearance and margins.

RESULTS AND DISCUSSION

The resistance profiles of uropathogens to antimicrobial agents used to treat UTI have been documented in several recent studies[13,18]. The focus of the present study is to clearly define the patients tested positive for UTI with uncomplicated community-acquired UTI. Antibiotic susceptibility testing and ESBL detection were carried out according to the protocols defined by the clinical and laboratory standards institute (CLSI) criteria. A total of eighteen antibiotics and the susceptibility of the test organisms to these antibiotics were determined in this study.

Isolation and characterization of microbes from clinical specimens

The pure isolates tested positive for UTI were inoculated on EMB agar plates and the colonies forming green metallic sheen were observed and enumerated. Similarly growth on MSA agar plates and formation of golden yellow, smooth colonies along with the production of acid which turns the media colour yellow were observed and enumerated. On PIA plates showed bluish-green, pigmented colonies were observed. Shiny pink colour colonies on MacConkey agar were observed and recorded.

Characterization of isolates

The clinical isolates were characterized on the basis of microscopic and biochemical tests performed according to standard procedures. The isolate was cultured on different growth and differential media to study the growth and morphological characteristics.

Morphological appearance of multidrug resistant bacterial isolates

The isolates were further characterized by standard microbiological and biochemical tests. Gram's staining of the samples was performed and it was found that the isolates were Gram negative rods and the growth on nutrient agar showed circular, greyish white mucoid colonies in the range of 2-3 mm. Cultivation of the isolates in MacConkey agar showed large shiny colonies which were dark pink in colour with mucoid appearance (Fig. 1, Table 1). The organism was found to be a lactose fermenter, and the pink colour of the colonies was due to the formation of pink pigment formed by the lactose sugar fermentation.

Microscopic observation of *K. pneumoniae* isolate

The colonies were isolated on the basis of the clear zone, morphology and biochemical tests and, Gram's staining was performed on selected isolates. Gram's staining of the smear of *K.*

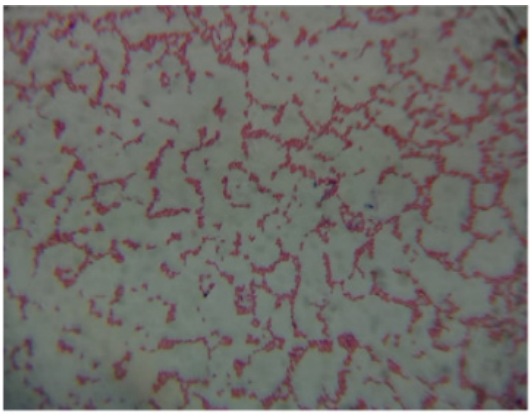

Fig. 2. Gram's stained slide of *K. pneumoniae*

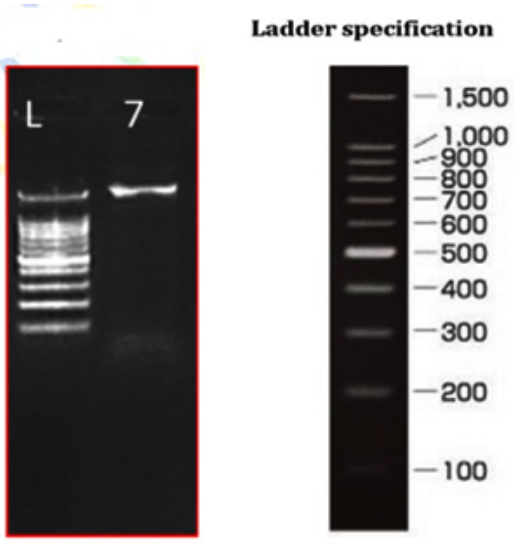

Fig. 3. Electrophoretic Chromatogram of the 16S DNA isolated from *K. pneumoniae*

pneumoniae showed the presence of pink colored rods, confirming the isolate as gram negative bacilli (Fig. 2.) Further characterization of the isolates was carried out by conducting different Biochemical tests.

Biochemical tests for identification of urinary sample isolates

The morphological and biochemical tests confirm that the organism is *Klebsiella pneumoniae.* The isolate was further characterized by performing biochemical tests and the isolate showed a negative test for indole and methyl red, positive for vogue's-proskauer test. The isolate was a catalase positive organism. The isolate showed no oxidase activity and H_2S production (Table 2 and 3).

The isolates were capable of fermenting all the sugars except DNA and erythritol (Table 3).

Klebsiella infections are the most common nosocomial infections in hospitals and are usually spread through person-to-person contact. *Klebsiella* infections spread very easily and rapidly. Hospitals and health care facilities are the most common places of *Klebsiella,* due to the distinct features of the bacteria. *K. pneumoniae* is reported to be responsible for 6-17% of UTI's worldwide. Lipopolysaccharide (LPS) and capsular polysaccharide (CPS) are the two foremost important virulence factors of *K. pneumoniae* which are causative factors for sepsis. The pathogen produces LPS contains lipid A, core, and O-polysaccharide antigen to inhibit the complement-mediated killing by antimicrobial agents. The outer layer of the pathogen containing polymorph nuclear cells is the CPS and is responsible for generating resistance against

Fig. 4. Sequence alignment of *K. pneumoniae* with other strains

phagocytosis. The CPS reduces interaction between bacterial cells through reducing the quantity of C3 being placed on the bacteria by acting as a contact barrier to obstruct contact between macrophage receptors and their ligands on the bacterial surface.

CPS is very critical to modulate the interaction between surfactant protein D (SP-D) and *K. pneumoniae*. SP-D improves phagocytic clearance by human alveolar macrophages by mediating aggregation. CPS prevents the attachment of C3 and SP-D leading to clearance of the microbe from the lower respiratory tract, resulting in pneumonia[19]. *K. pneumoniae* utilises the capsular antigens which are composed of complex acidic polysaccharides essential to the virulence to this pathogenic organism. The capsular antigens are classified into 77 serological types and are usually made up of uronic acids. For protection against cellular immunity, the bacterium utilizes thick bundles of fibrillose structures to evade phagocytosis by polymorph nuclear granulocytes. This mechanism also helps in protecting the pathogen being killing by bactericidal serum factors and also inhibits complement constituents, like C3b, which inactivate the pathogen by opsonisation[2]. These effective virulence factors

Description	Max Score	Total Score	Query Coverage	E Value	Identity	Accession
Klebsiella pneumoniae strain ZJ-01 16S ribosomal RNA gene, partial sequence	2501	2501	99%	0	98%	KF974479.1
Klebsiella pneumoniae strain WA-1 16S ribosomal RNA gene, partial sequence	2499	2499	99%	0	98%	MH045825.1
Klebsiella sp. CCFM8380 16S ribosomal RNA gene, partial sequence	2499	2499	99%	0	98%	KJ803937.1
Klebsiella pneumoniae subsp. pneumoniae HS11286, complete genome	2499	19906	99%	0	98%	CP003200.1
Klebsiella pneumoniae strain ZS685683 16S ribosomal RNA gene, partial sequence	2495	2495	99%	0	98%	MH077539.1
Klebsiella pneumoniae strain ZG19 16S ribosomal RNA gene, partial sequence	2495	2495	99%	0	98%	MG859655.1
Klebsiella pneumoniae strain QLR5-1 16S ribosomal RNA gene, partial sequence	2495	2495	99%	0	98%	MF767576.1
Klebsiella pneumoniae strain QLR8-2 16S ribosomal RNA gene, partial sequence	2495	2495	99%	0	98%	MF767575.1
Klebsiella pneumoniae strain QLR-8 16S ribosomal RNA gene, partial sequence	2495	2495	99%	0	98%	KM096437.1
Klebsiella pneumoniae strain QLR-5 16S ribosomal RNA gene, partial sequence	2495	2495	99%	0	98%	KM096436.1

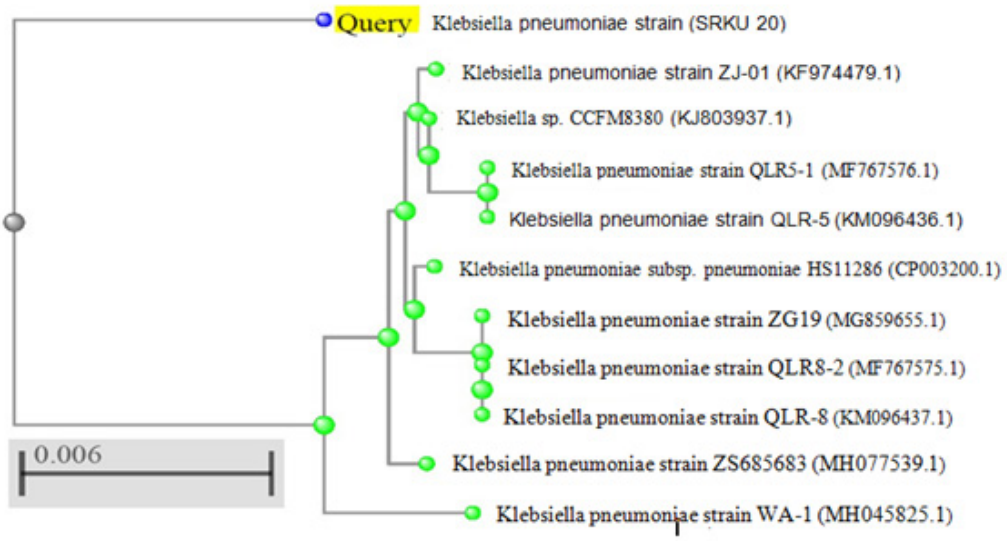

Fig. 5. Phylogenetic tree of *Klebsiella pneumoniae*

were found to be powerful enough to inhibit the differentiation capacity of macrophages *in vitro*.

Molecular characterization of the EMDR isolate *Klebsiella pneumoniae*

The isolate *Klebsiella pneumoniae* subjected to genetic analysis and the 16S DNA was sequenced by Sanger Sequencing Technique.

Post sequence analysis and tree formation

A table of closely related sequences of similar organisms was generated along with the sequence of the test organism. Organisms with similar sequences were selected in FASTA format, and BLAST search for sequence similarity was checked for multiple sequence alignment (MSA) using the alignment tool BLAST X and a phylogenetic tree (NCBI) was developed. with reference link https://www.ncbi.nlm.nih.gov/nuccore/MZ318713

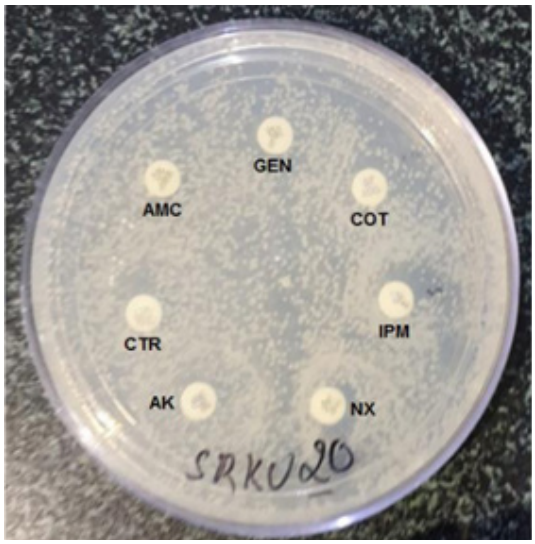

Fig. 6. Antibiotic Sensitivity test for *Klebsiella pneumoniae* isolate

Molecular identification results

Purity of the genomic DNA at A260nm/280nm was found to be 1.8 and the concentration of genomic DNA was 47.5 ng/il. The amplified sample was electrophoresed in 1.2% Agarose gel for 45 minutes and 70 volts. A single sharp 1151 bp amplified region of 16S DNA was visualized. The first lane of the gel was loaded with 100-1000 bp sequences as ladder and Lane 2 contained the amplified 1151 bp 16S region (Fig 3).

Partial gene 16S of 1151 bp was received after sequencing and a FASTA format of the same is shown below.

Sequence analysis

A gene with a 1151 bp length, partial 16S rRNA sequence in FASTA format was subjected to BLAST sequence search in GenBank and the BLAST result showed that the test organism was *Klebsiella pneumoniae* with 99% similarity to reference *Klebsiella penumoniae* strains and E value 0.003.

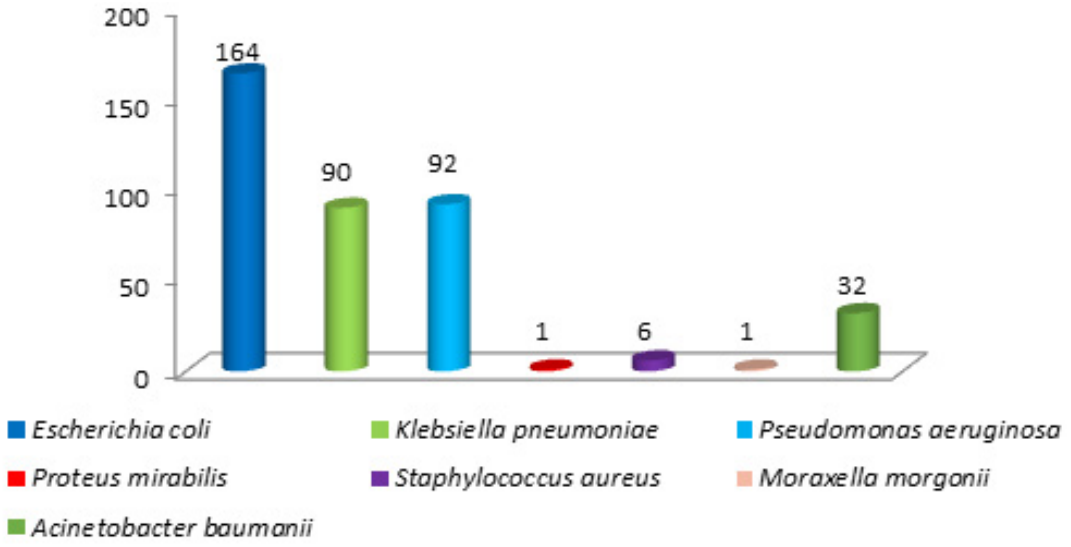

Fig. 7. Screening of urine samples presenting UTI among pregnant women

RID : 8GSU2SW5013
Program : BLASTN
Database :nt
Query ID :lcl|Query_9205
Description: None
Molecule type: dna
Query Length: 1151

Forward sequence data

AGKSSGGCAGGCTACACATGCAAGT
CGAGCGGTAGCACAGAGAGCTTGCTCTCG
GG TGACGAGCGGCGGACGGGTGAGTAA
TGTCTGGGAAACTGCCTGATGGAG
GGGGATAACTACTGGAAACGGTAGCT
AATACCGCATAAYGTCGCAAGACC
AAAGTGGGGGACCTTCGGGCC
TCATGCCATCAGATGTGCCCAGATGGGA
TTAGCTAGTAGGTGGGGTAACGGCT
CACC TAGGCGACGATCCCTAGCTGGTCTG
AGAGGATGACCAGCCACACTGGAA
CTGAGACACGGTCCAGACTCCTACGG
GAGGCAGCAGTGGGGAATATTGC
ACAATGGGCGCAAGCCTGATGC
AGCCATGCCGCGTGTGTGAAGAAGGCCT
TCGGGTTGTAAAGCACTTTCAGCGG
GGAGGAAGGCGKTRAGGTTAATAA
CCTYRYCGATTGACGTTACCCGCAGAA
GAAGCACCGGCTAACTCCGTGC
CAGCAGCCGCGGTAATACGGAGGGTGC
AAGCGTTAATCGGAATTACTGG
GCGTAAAGCGCACGCAGGCGG TCTGTC
AAGTCGGATGTGAAATCCCCGGG
CTYAACCTGGGAACTGCATTCGAAAC
TGGCAGGCTAGAGTCTTGTAGAGGG
GGGTAGAATTCCAGGTGTAGCGGTGA
AATGCGTAGAGATCTGGAGGAATACC
GGTGGCGAAGGCGGCCCCCTGGACAA
AGACTGACGCTCAGGTGCGAAAGCGTGG
GGAGCAAACAGGATTAGATACCCTGGT
AGTCCACGCCGTAAACGATGTCGATTT
GGAGGTTGTGCCCTTGAGGCGTGGCT
TCCGGAGCTAACGCGTTAAATCGACC
CCTGGGGAGTACGGCCGCA

Table 1. Colony morphology of microbial isolates isolated for screened antibiotic resistance

No.	Sample ID	Gram's Stain	Shape	Nutrient agar	MacConkey Agar
1	*K. pneumoniae*	Negative	Rod	Circular, Greyish white mucoid colonies (2-3mm)	Large shinny and dark pink in color with mucoid colonies (2-3mm)

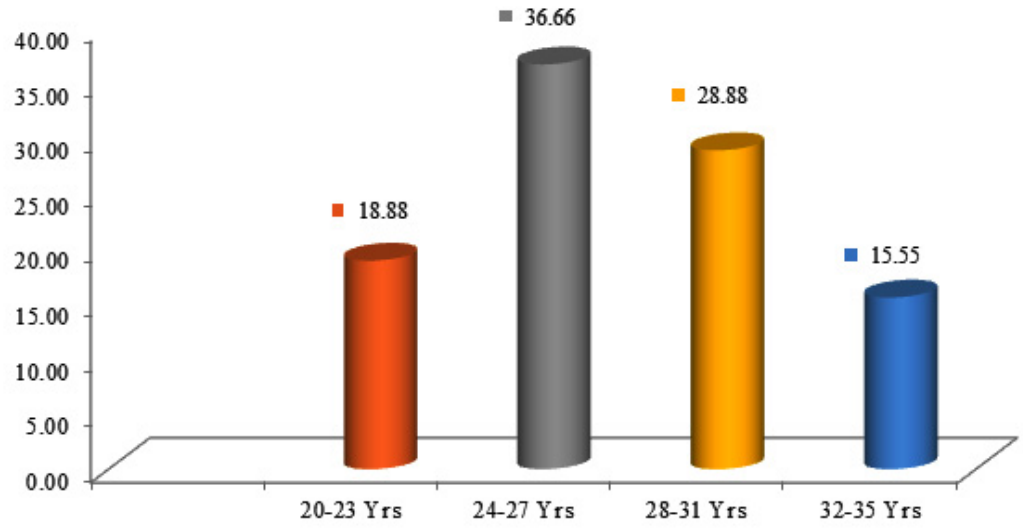

Fig. 8. Age wise prevalence of *K. pneumoniae* UTIs among pregnant women

Table 2. Biochemical tests for the characterization of *Klebseilla pneumoniae.*

No.	Characteristics	Result
1	Capsule	Positive
2	Flagella	Negative
3	Motility	Negative
4	Pigment	Negative
5	Shape	Rod
6	Spore	Negative
7	Gram's Staining	Negative
8	Gas	Positive
9	Gelatin Hydrolysis	Negative
10	Grown in KCN	Positive
11	H_2S Production	Negative
12	Catalase	Positive
13	Citrate	Positive
14	Indole	Negative
15	Methyl Red	Negative
16	MUG Test	Positive
17	Nitrate Reduction	Positive
18	Oxidative Ferment	Fermentative
19	Oxydase	Negative
20	TSIA	Alkaline / Acid
21	Urease	Positive
22	Voges-Proskauer	Positive
23	Adenolol	Positive
24	Arabinose	Positive
25	Arabitol	Positive
26	Cellobiose	Positive
27	Dnase	Negative
28	Erythrytol	Negative
29	Esculin hydrolysis	Positive
30	Glucose	Positive
31	Glycerol	Positive
32	Inositol	Positive
33	Lactose	Positive
34	Maltose	Positive
35	Mannitol	Positive
36	Melibiose	Positive
37	Mucate	Positive
38	Mannose	Positive
39	Myoinositol	Positive
40	Raffinose	Positive
41	Rhamnose	Positive
42	Salicin	Positive
43	Sorbitol	Positive
44	Tartarate	Positive
45	Xylose	Positive

A G G T T A A A A C T C A A A T G A A T T G
A C G G G G G C C C G C A C A A G C G G T
G G A G C A T G T G G T T T A A T T
C G A T G C A A C G C G A A G A A C C T
TACCTGGTCTTGACATCCACAGAACTTWC
C A G A G A T G C W T T G G T G C C T T C
GGGAACTGTGAGACAGGTGCTGCATG
GCTGTCGTCAGCTCGTGTTGTGAATGTTG
G G T A G T C C C G C A C G A G C G C A A C
C C T T A T C T T G T G C A G C G G A T C A G G C C
GGCAACTCAAAGCAGACTGGCAGTGATA
ACTGGCAGAAGGTGGGGGATKAACGTCA

Reverse sequence data

C T A Y M A A A G T G G T A G C G C C C T
C C C G A A G G T T A A G C T A C C T A C T T C T T
T T G C A A C C C A C T C C C A T G G T G T G A C G G G C G
G T G T G T A C A A G G C C C G G G A A C G T A T
T C A C C G T A G C A T T C T G A T C T A C G A T T A
C T A G C G A T T C C G A C T T C A T G G A G T C G A G T T
G C A G A C T C C A A T C C G G A C T A C G A
C A T A C T T T A T G A G G T C C G C T T G C T C T C G C G
A G G T C G C T T C T C T T T G T A T A T G C C A T T G
T A G C A C G T G T G T A G C C C T G G T C G T A A G G G
C C A T G A T G A C T T G A C G T C A T C C C C
A C C T T C C T C C A G T T T A T C A C T G G C A G T
C T C C T T T G A G T T C C C G G C C K R A C C G C T
G G C A A C A A A G G A T A A G G G T T G C G C T C G
T T G C G G G A C T T A A C C C A A C A T T T C
A C A A C A C G A G C T G A C G A C A G C C A T G C
A G C A C C T G T C T C A C A G T T C C C G A A
G G C A C C A A W S C A T C T C T G S W A A G T T C T G
T G G A T G T C A A G A C C A G G T A
A G G T T C T T C G C G T T G C A T C G A A T T A
A A C C A C A T G C T C C A C C G C T T G T G C G G G C
C C C C G T C A A T T C A T T T G A G T T T T A A C
C T T G C G G C C G T A C T C C C C A G G C G G T C G A T
T T A A C G C G T T A G C T C C G G A A G C
C A C G C C T C A A G G G C A C A A C C T C C A A
A T C G A C A T C G T T T A C G G C G T G G A C T A C
C A G G G T A T C T A A T C C T G T T T G C T C C C C A
C G C T T T C G C A C C T G A G C G T C A G T C T T T
G T C C A G G G G G C C G C C T T C G C C A C C G G T A T T
C C T C C A G A T C T C T A C G C A T T T C A C C G C T A
C A C C T G G A A T T C T A C C C C C C T C T A C A
A G A C T C T A G C C T G C C A G T T T C
G A A T G C A G T T C C C A G G T T G A G C C
C G G G G A T T T C A C A T C C G A C T T G A C A
G A C C G C C T G C G T G C G C T T T A C G C C C
A G T A A T T C C G A T T A A C G C T T G C A C C C T C C G
T A T T A C C G C G G C T G C T G G C A C G G A G T T A

GCCGGTGCTTCTTCTGCGGGTAACGT CAATCGMYGAGGTTATTAACCTCAC GCCTTCCTCCCCGCTGAAAGTGCTTT ACACCCGAC CTTCTTCACACACGCCGCATG C C T G C A T C A G C T G C G C C A A T G T GCATATCCCACTGCTGCATCCCGCTA GATCTGATCGGTATCTCAGCTATCA.

The strain exhibited very high sequence similarity with other pathogenic *K. pneumonia,* which are highly resistant to antibiotics (Fig. 4).

Figure 5 represents the phylogenetic tree of *K. pneumoniae* and it is confirmed the strain is a pathogenic *Klebsiella pneumoniae*. It belongs to the enterobacteriae and compares with very high sequence similarity to the strains KJ803937, CP003200, MF767576, KM096437, KF974479 and MG859655 which are reported uropathogens isolated from several hospital samples in different studies and the genetic sequences submitted to genbank (MZ318713) with the accession numbers. These results confirm that the organism is *Klebsiella pneumoniae*.

Screening of samples for antibiotic resistance

A total of 2011 samples were screened for antibiotic resistant microbes in Hospital Samples. 386 samples tested positive for antibiotic resistance and 1625 samples tested negative.

Antibiotic sensitivity test for *Klebsiella pneumoniae* isolate

The isolate *K. pneumoniae* showed an increased resistance to major types of antimicrobial agents which included ß-lactams (third- and fourth-generation cephalosporins), aminoglycosides

Table 3. Screening of antibiotic resistance among clinical isolates of *K. pneumoniae*

No	Antibiotic	Abbreviation	Susceptibility (R*/S**)	Zone of Inhibition (mm)
1	Piperacillin	PI	S	20
2	Amoxycilline	AMC	R	7
3	Ceftriaxozone	CTR	R	6
4	Cephalexin	CN	R	8
5	Norfloxacin	NX	R	7
6	Levofloxacin	LE	S	21
7	Amikacin	AK	R	6
8	Pipericillin Tazobactum	PIT	S	23
9	Gentamycin	GEN	R	9
10	Imipenem	IPM	R	6
11	Meropenem	MRP	R	9
12	Colistin	CL	S	15
13	Linezolid	LZ	S	24
14	Co-Trimoxazole	COT	R	8
15	Nitrofurantoin	NIT	S	18
16	Vancomycin	VA	S	18

*R= Resistant **S= Sensitive

Table 4. Incidence of *K. pneumoniae* implicated in urinary tract infection throughout the study period

Age Group		20-23 Years	24-27 Years	28-31 Years	32-35 Years
Name of Isolate	No of Isolates	Total(n=17)	Total(n= 33)	Total(n=26)	Total (n=14)
Klebsiella pneumoniae	90	17 (18.88%)	33 (36.66%)	26 (28.88%)	14 (15.55%)
Total of UTI (%)	386 (N)	20.98	37.05	21.5	20.47

Figures in parenthesis indicate Percentage determined in relation to N.
The incidence of UTI causing microbes by age group.
n total number of individual bacterial isolate for each age group;

& fluoroquinolones (Table 4.), Amoxycillin, Ceftriaxazone, Cephalexin, Norfloxacin, Levofloxacin, Amikacin, Piperacillin-Tazobactum, Gentamycin, Imepenem, Meropenem, Colistin, Linezolid, Sulfamethaxzole-Trimethoprim, Nitrofurantoin, Vancomycin. Screening for ESBL and confirmation tests were carried out by the disc diffusion technique according to the Clinical Laboratory Standard Institute (CLSI-2019) criteria.

The isolates of *K. pneumoniae* were found to be ESBL producers and showed high-level of resistance for quinolones, aminoglycoside, carbapenems and cotrimoxazole and the isolates were found to be susceptible to piperacillin and levofloxacin (Table 4, Fig 6).

The prevalence of ESBL producing *Klebsiella pneumonia* among the total UTI positive isolates was *23.31%.*

Klebsiella pneumoniae is reported to be extensively resistant to antibiotics. The organism is sensitive to only towards colistin, linezolid, nitrofurantoin vancomycin and piperacillin-tazobactam. *K. pneumoniae* is the most common causative organism for community acquired infections and recently it is most commonly found to be the major cause of hospital acquired pathogens (HAP). The predominant mechanism of resistance to antibiotics in *K. Pneumonia* is through the production of enzymes such as Extended Spectrum β-Lactamase (ESBLs) and carbapenamase[7,20,21], and this is more prominent in *K. pneumoniae* compared to other organisms.

Over exposure to antibiotics is the most important risk factor of multi drug resistance (MDR) in *K. pneumoniae.* Highly intensive and prolonged use of antibiotics in hospitals is the main contributor for the emergence and spread of highly resistant bacteria for health-care associated infections (HAIs)[22]. *K. pneumoniae* is known to play an important role in spreading antimicrobial resistance genes from environmental contaminant bacteria to clinically important bacteria[23]. *K. pneumoniae* was resistant to various antibiotics in most of the cases, and ampicillin, cefazolin, and cefuroxime were found to be least effective for *K. pneumonia*. The antibiotics amikacin, piperacillin-tazobactam, and meropenem were found to be highly effective in inhibiting the pathogen. Similar findings were reported by Cepas *et.al.* (2019)[24] wherein they have reported 40% resistance of *K. pneumoniae* strains to ciprofloxacin. In the present study it was found that the highest resistance to antibiotics was against Ampicillin, Piperacillin, Trimethoprim and Gentamicin and lowest resistance was against Imipenem and Chloramphenicol. The results of the study are similar to those reported by Parsaie Mehr *et.al.*,[25] who reported the resistance of *K. pneumoniae* isolates which isolated from several hospitals in Korea and it was found that the organism was resistant to Imepenem. Also our results are in agreement with those of Vasaikar *et. al.* (2017)[26] who reported the resistance of *K. pneumoniae* isolates from several hospitals in South Africa for Ciprofloxacin, Gentamicin and Sulfamethoxazole Trimethoprim. Our results are also consistent with the findings of Parsaie Mehr *et.al.* (2017)[25] who reported the resistance to Chloramphenicol also. The results of this study also were found to be similar to findings of Ghasemian *et al*. (2017)[18] who reported that isolates of *K. pneumonia* from several hospitals in Tehran were highly resistant to Cefotaxime.

The underlying phenomenon for increasing antibiotic resistance in *K. pneumoniae* was attributed to the resistome evolving under continuous antibiotic selective pressure leading to accumulation of various antibiotic resistance genes (ARGs) in *K. pneumoniae* genetic material leading to evolution of multidrug resistance (MDR) and extremely drug resistance (XDR) carbapenem-resistant strains. The self-transferrable plasmids are known as *K. pneumoniae* 'mobilome' which include plasmids that harbour ARGs and transposons. This diverse resistome and mobilome of *K. pneumoniae* in known to contribute to the evolution of the extremely drug resistance (XDR) phenotype in both epidemic and sporadic sequence types (STs) or clones[25]. When these ARGs and plasmids become associated with strains that have high epidemic potential, the clones become HiR2[22,27,28].

Screening urine samples for presence of UTI among pregnant women

From among 2011 samples collected during the study period, 386 samples tested positive for UTI and 90 samples accounted to *K. pneumoniae* infections (Fig 7).

The other predominant isolates belonged to *E. coli, Pseudomonas*

aeruginosa and *Acinetobacter baumanii.* However, owing to the high risk of infection by *K. pneumoniae,* the isolates were further considered for this study.

Prevalence of antimicrobial resistance in *K. pneumoniae* isolates from pregnant women patients

The isolated samples were subjected to antibiotic susceptibility testing by the Kirby– Bauer disc diffusion method as per Clinical Laboratory Standard Institute standards for antimicrobial testing. It was found that the highest incidence of *K. pneumoniae* infections occurred among women in the age group of 24-27 years (36.66%) followed by 28-31 years (28.88%) (Table 5, Fig 8). The probable reason for the high incidence might be attributed to the active reproductive age and prevalent life style of the subjects.

In the present study, the urine samples were inoculated on selective media such as EMB, CLED, MSA, PIA, CIA followed by biochemical tests which resulted in the isolation of *K. pneumonia* uropathogens and antibiotic susceptibility tests determined that *K. pneumoniae* was susceptible to piperacillin, levofloxacin, colistin, linezolid, nitrofurantoin and vancomycin, while it was resistant to amikacin, imipenem, amoxycilline, norfloxacin, Cephalexin and co-trimoxazole,. The isolates were moderately resistant to gentamycin and meropenem against *Klebsiella pneumonia*. ESBL producing organisms are the most common causative nosocomial pathogens, and it is very essential to detect and treat them as early as possible. The present study will provide the basis for routine screening of antibiotics against ESBL producing bacteria. The study will provide the basis for optimizing precise antibiotic therapies against specific bacterial cultures. Thus, the results will help in exploring the specific treatment against such pathogens borne infections.

CONCLUSIONS

We here by conclude that the above data shows that antibiotic resistance among hospital patients is increasing at a rapid pace due to the indiscriminate use of antibiotics and antimicrobial agents as a global problem. Strong surveillance, close monitoring and early detection of AMGs, MDR and XDR bacterial strains in all health care facilities to minimise the menace of multidrug resistant bacteria. Monitoring and reporting changes in isolates that are resistant to antibiotics is crucial for public healthcare organisations; this will help the doctors to prescribe the appropriate antibiotics. More cautious attempts should be made to develop a new line of antimicrobials. The domestic health officials in fields must coordinate a multifaceted plan as part of the control measures.

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Conflict of Interest

There is no conflict of Interest in this article.

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