

Isolation and Identification of Multidrug Resistant *Klebsiella* Spp. Causing Urinary Tract Infections

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ABSTRACT

Klebsiella pneumoniae is an important pathogen in hospitalized patients, contributing to their morbidity and mortality due to its multiple resistance mechanism. Therefore, as therapeutic options become restricted, the search for new agents is a priority. Lately an accelerated increase in frequency of multidrug resistant clinical strains has severely limited the availability of therapeutic options. In this study one hundred and fifty samples of urine were collected from patients admitted to Hawler teaching hospital, Rizgary hospital, and Par hospital, During period of September to March 2016. 10 isolated of *Klebsiella pneumoniae* were identified by using cultural, morphological, characteristics, biochemical test and Vitek 2 compact system. The highest percentage of *Klebsiella pneumoniae* in female 9 (5.2%) was higher than those in male patients 1(2.85%). Susceptibility of *Klebsiella pneumoniae* isolated to different antibiotics was examined, Methicillin 8 (80%), Imepenem 7 (70%) were the most effective antimicrobial agents against *Klebsiella pneumoniae* isolated and most of isolates showed that high resistance to Gentamicin 10(100%), followed by Ampicillin 9(90%) and Nitrofurantoin 9(90%) and Ciprofloxacin 9 (90%). On the other hand our results indicated that most isolates of were multiresistance which showed resistance to >4 antibiotics. The problem of antimicrobial resistance in bacterial pathogens has been fairly described as a growing global crisis, Rates of reported resistance in common pathogens are reaching levels in many corners of the world that preclude empirical use of many of our most potent and reliable antimicrobial agents.

Keywords: *Klebsiella* species, wound infection, antimicrobial resistance

INTRODUCTION

Bacteria that belong to the genus *Klebsiella* are facultative, anaerobic, non motile, Gram-negative rods that possess a prominent polysaccharide capsule [1]. *Klebsiella* species exist as normal flora in the gastrointestinal tract of animals and humans [2]. Despite this, *Klebsiella* species can cause severe infections that include meningitis, bronchitis, bacteremia, pneumonia, urinary tract infections in humans and animals [3]. In humans these infections are common in patients who are admitted in hospitals and those who are immune compromised. Thus most infections caused by *Klebsiella* species result from consumption of contaminated food such as rotten fish and/or water [4]. *Klebsiella* express two types of cell surface antigens viz; the O and K antigens that contribute to pathogenicity in these species [1]. *Klebsiella* a normal member of the gastrointestinal tract flora, has emerged as a significant nosocomial pathogen in neonatal units, Nosocomial *Klebsiella* infections are also remarkably troublesome, particularly in

premature infants and intensive care units (ICUs). Pediatric patients are easily colonized by *Klebsiella* spp. Intestinal and oropharyngeal colonization acts as the main reservoir for nosocomial outbreaks. In fact, *K. pneumoniae* has been reported as a prominent cause of infections in individuals with indwelling urinary catheters [5].

In humans, they may colonize the skin, pharynx, or gastrointestinal tract. They may also colonize sterile wounds and urine [6]. Striking clinical finding concerning a new manifestation of community-acquired *K. pneumoniae* infections has been documented [7]. An unusual invasive presentation of *K. pneumoniae* infection, primary bacteremic liver abscess, has been described by numerous investigators in Asia [8]. In the past decade, the emergence of extended-spectrum beta-lactamases in *K. pneumoniae* strains and their dissemination have greatly complicated chemotherapy, and outbreak due to ESBL-producing organisms have been reported in several countries [9]. Each year UTI cause losses in work time, morbidity and medical

costs. For people living in long-term health care settings the incidence of UTI might be as high as 50%. Bacteriuria is the presence of bacteria in the urine and it is well known that urine is free of bacteria. Bacteriuria can be asymptomatic or symptomatic and it doesn't necessarily mean a urinary tract infection. The majority of pathogens that cause UTI are either coli forms or enterococci. Coliforms include enterobacteriaceae family members of *E. coli*, *Enterobacter* and *Klebsiella*. *E. coli* is a bacteria inhabitant in the intestine that usually live in human without causing any problem. It makes no distinction for age and accounts for the majority (80-90%) of uncomplicated infections in young and old [10].

Extensive use of broad-spectrum antibiotics in hospitalized patients has led to both increased carriage of *Klebsiella* and, subsequently, the development of multidrug-resistant strains that produce extended-spectrum beta-lactamase (ESBL) [9]. These strains are highly virulent, show capsular type K55, and have an extraordinary ability to spread [11]. Most outbreaks are due to a single clone or single gene the bowel is the major site of colonization with infection of the urinary tract, respiratory tract, and wounds [12]. Extensive use of broad-spectrum antibiotics in hospitalized patients has led to both increased carriage of *Klebsiella* and, subsequently, the development of multidrug-resistant strains that produce extended-spectrum beta-lactamase

(Paterson, 2000; Kaye et al., 2000). These strains are highly virulent, show capsular type K55, and have an extraordinary ability to spread (Kaye 2000, Mitford, 2008, Adams-Haduch et al., 2009). Most outbreaks are due to a single clone or single gene (Kaye, 2000); the bowel is the major site of colonization with infection of the urinary tract, respiratory tract, and wounds (Kaye, 2000). Extensive use of broad-spectrum antibiotics in hospitalized patients has led to both increased carriage of *Klebsiella* and, subsequently, the development of multidrug-resistant strains that produce extended-spectrum beta-lactamase (ESBL) (Paterson, 2000; Kaye et al., 2000). These strains are highly virulent, show capsular type K55, and have an extraordinary ability to spread (Kaye 2000, Mitford, 2008, Adams-Haduch et al., 2009). Most outbreaks are due to a single clone or single gene (Kaye, 2000); the bowel is the major site of colonization with infection of the urinary tract, respiratory tract, and wounds (Kaye, 2000).

Extensive use of broad-spectrum antibiotics in hospitalized patients has led to both increased carriage of *Klebsiella* and, subsequently, the development of multidrug-resistant strains that produce extended-spectrum beta-lactamase. These strains are highly virulent, show capsular type K55, and have an extraordinary ability to spread [11]. Multidrug resistant bacteria cause serious nosocomial and community acquired infections that are hard to eradicate by using available antibiotics. B-lactam antibiotics disturb peptide bond formation by acting as competitive inhibitors to these PBPs. These result in formation of irreversible covalent bonded penicilloyl-enzyme complexes with weak cross-linked peptidoglycans, thus ease bacteria lyses and death [13].

METHODS

Samples Collection

A total of 150 urine sample were collected from patients suffering from Urinary Tract Infection from both sexes (males and females) attending public hospitals. A positive urine culture is based on the growth of bacteria at high number of colony forming units (CFU). Urine culture results should be interpreted in conjunction with clinical symptoms of UTI. For clean-catch urine samples, a positive urine culture as indicated by the growth of bacteria greater than 100,000 CFUs/ml is suggestive of UTI. Liability of results is determined by the quality of the specimen collection, transport, handling to the laboratory [14]. For isolation of microorganisms, the specimen of vaginal swab was directly inoculated on culture media Blood agar and MacConkey agar plates were incubated aerobically at 37°C for 24-48 hours. Pure colonies of isolated microorganisms were identified using morphological, biochemical tests including API system [15]. Species identification and antibiograms for pathogens were performed using Vitek 2 compact system [16].

Vitek 2 Compact System

The newly redesigned colorimetric Vitek 2 compact system, with updated advanced expert system (AES) (bioMérieux, Marcy l'Etoile, France) was evaluated for its accuracy and rapidity to identify clinical isolates and to detect several antimicrobial resistances [17]. Principles of the Vitek 2 is an automated microbiology system utilizing growth-based technology. This system accommodates the colorimetric reagent cards that are incubated and

interpreted automatically. Overall, the Vitek 2 gave 95.8% of compatibility with the reference API strips (bioMerieux) in the identifications (ID)s of the Gram- positive cocci (GPC), Gram-negative rods (GNR), and yeasts. The accuracy was finally estimated to 98.3% through additional confirmatory tests. Also, > 90% of identifications of GPC and GNR were obtained within 7 hours of incubation. The most resistant isolates were identified within 12 hours of incubation. In conclusion, the new colorimetric Vitek 2 compact system with AES greatly improved its accuracy in species identification and detection of antimicrobial resistances, and it will be highly acceptable to clinical microbiology laboratory function [18].

Antimicrobial Susceptibility Test By Vitek2 System

The system includes an AES that analyzes minimum inhibitory concentration (MIC) patterns and detects phenotypes for most organisms tested. This helps optimize laboratory efficiency for lean laboratory management. Rapid results allow clinicians to discontinue empiric therapy and prescribe targeted therapy, resulting in improved patient outcomes and enhanced antibiotic stewardship. With its ability to provide accurate "fingerprint" recognition of bacterial resistance mechanisms and phenotypes, the AES is a critical component of Vitek 2 technology. The Vitek 2 card contains 64 microwells. Each well contains identification substrates or antimicrobial. Vitek 2 offers a comprehensive menu for the identification and antibiotic susceptibility testing of organisms [19]. The Vitek 2 test card is sealed, which minimizes aerosols, spills, and personal contamination. Disposable waste is reduced by more than 80% over microtiter methods.

Antimicrobial Susceptibility Testing

Regarding antibiotic resistance pattern in *K. pneumoniae* isolates. Ten isolates of *K. pneumoniae* were screened for their susceptibility to 11 used antibiotics (Amikacine, Ampicillin, Methicillin, Ceftriaxone, Ciprofloxacin, Rifampicin, Gentamicin, Imepenem, Meropenem, Ampiclox, Nitrofurantoin). Disc diffusion method, also known as the Kirby-Bauer method was carried out according to the Clinical and Laboratory Standard Institute guidelines (CLSI), formerly the National Committee for Clinical Laboratory Standards (NCCLS) [20].

1. Muller Hinton agar was prepared and sterilized then the medium was cooled to 45°C and poured to sterilized Petri dish.
2. Muller Hinton agar containing 5% blood is used for testing Streptococci.
3. Inoculum suspension was prepared using colony suspension standardized to match the turbidity of a McFarland 0.5 standard.
4. The plates were inoculated by a sterile cotton swab dipped in bacterial suspension, then swabbed evenly across the surface of a Muller Hinton agar plates.
5. Sterile forceps was used to place the antibiotic disc on the surface of the inoculum.
6. The inoculated plates were incubated at 37°C for 18-24 hours.
7. After incubation the diameters of inhibition zone produced by antimicrobial inhibition of bacterial growth were measured using a ruler.
8. Each inhibition zone interpreted chart table which is recommended by NCCLS.

RESULTS AND DISCUSSION

Frequency of *Klebsiella pneumoniae* Isolated from Urinary Tract Infection

A total of (150) samples were collected from patients attending Hawler teaching hospital, Rizgary hospital, PAR hospital in Erbil city, our results showed that among (150) samples 10 were culture positive with *K. pneumoniae* while 140 were culture negative as in table (1). Out of (150) isolates of *K. pneumoniae* isolated from one source of infection from urinary tract infection. The positive cultures of *K. pneumoniae* were 10(6.67) isolates. These results were lower than with findings of Idomir *et al.*, [21]. from Romania who found were (63.6%) isolated from urine, (10.4%) from wound and (10%) from sputum. Of the many species within the *K. pneumoniae* is considered the most medically important *Klebsiella* species, causing 75% to 86% of clinical *Klebsiella* infections, with the next most commonly recovered species being *K. oxytoca*, which accounts for 13% to 25% of infections [22]. The presence of this bacteria in large numbers present in UTI might be attributed to the fact that these bacteria are often part of the resident flora and different virulence factors contributing to their pathogenicity and the difference in the result with others might be attributed to the number of taken sample size and the difference in the time of the study.

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Table1. Frequency of *K.pneumonia* isolated from urinary tract infections .

Patient	Number	%
Infected	10	6.67
Uninfected	140	93.33
Total	150	100

Relation between *K.Pneumoniae* with Gender (Female , Male)

Out of 150 samples ,10 samples were culture positive for *K.pneumoniae* ,the highest percentage of *K.pneumonia* in female 9 (5.2%) , Was higher than those in male patients 1 (2.85%).The highest percentage of infection by *K.pneumonia* in female 9(5.2%) were higher than those in male patients 1(2.85%).These results were agree with Hajar, [23]. from Iran who found that the incidence of *Klebsiella spp.* isolates was more prevalent in female 43 patients than in male 12 patients, but higher than our results.

Table2. Relation between *K.pnumoniae* with gender

Patients	Culture positive		Culture negative		Total	
	No.	%	No.	%	No.	%
Male	1	2.85	34	22.67	35	23.33
Female	9	5.2	106	70.67	115	76.7
Total	10	6.67	140	93.33	150	100

Antimicrobial Susceptibility Testing

All *K.pneumonia* isolated were tested for antimicrobial susceptibility testing testing and the results were interpreted according to standard value provided by clinical and laboratory standard institute of antimicrobial sensitivity testing as shown in table (3), The most sensitive antibiotics were Methicillin 8

Table3. Antimicrobial susceptibility testing for *K.pneumoniae* .

Antibiotic	GM	CIP	IPM	APM	INN	NIT	RIF	MEM	CRO	AM	AK
No.f Resistant Isolates	10	8	3	9	2	9	8	5	7	9	7
Resistance %	100	80	30	90	20	90	80	50	70	90	70

AK:Amikacine, AM:Ampicillin, INN:Methicillin, CRO:Ceftriaxone, CIP:Ciprofloxacin, RIF:Rifampicin, GM:Gentamicin, IPM:Imepenen, MEM:Meropenem, AMP:Ampiclox , NIT:Nitrofurantoin .

The Number of Antibiotics (sensitive and resistant) for *K.pneumoniae*

In current study all isolates of *K.pneumonia* isolated from urinary tract infection samples were multi resistance to six or more antibiotics while one isolates was resistance to all antibiotics.

The observed changes in this study have serious implications as most clinicians treat patients without recourse to laboratory guidance. Such treatments are usually based on known

(80%) , Imepenen 7 (70%) ,while the most resistance antibiotics were GM Gentamicin 10(100%) ,followed by Ampicillin 9(90%) and Nitrofurantoin 9(90%) and Ciprofloxacin 9 (90%). Results indicate that most effective antibiotic were Methicillin 80%, followed by Imipenem 70% as in table (3) which was similar to the founding of Gupta *et al*[24]. . While the less effective antibiotics with highest resistant percentage (100%) was to Gentamicin followed by Ampicillin(90%),Amipiclox(90%). In vitro data showed a wide range of beta-lactams, aminoglycosides, quinolones and other antibiotics are useful for treatment of *Klebsiellae* infections [25]. The clinical isolates of *K.pneumoniae* were tested for antimicrobial sensitivity and most of them were found to be multidrug resistant. All the *Klebsiella pneumoniae* isolates were resistant to Gentamycin . In such cases the disease is prone to progress to permanent debilitation or death of the patient if, isolation and identification of the causative agent and the subsequent antimicrobial susceptibility testing is not carried out at the early stage of the disease [26]. It is note worthy to mention that spreading of resistant *of K.pneumonia* in the last few decades is incredibly increased which limits the choice of the therapeutic option for the treatment of infection caused by this microorganism. Clinicians also frequently commence patients on antibiotic therapy before sending samples to the microbiology laboratory such that many of the samples are negative when cultured. Abuse and misuse of antibiotics have been known to contribute to the development of antibiotic resistance.

aetiological agents and susceptibilities. This observed change in the prevalence of uropathogens may lead to change in antimicrobial susceptibility and ineffective treatment. Therefore, clinicians should rely on laboratory guidance before therapy as this will overcome the problem of mistreatment and reduce the emergence of resistant uropathogens [27]. The indiscriminate use of antimicrobials over prolonged periods has led to the emergence of multi drug resistant (MDR) strains. Whenever a new and effective antibiotic is introduced,

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bacteria after exposure to this antimicrobial, acquire resistance through different mechanism. As shown in table (4) , most of *K.pneumoniae*

isolated from urine samples were multi resistance to more than 6 antibiotics while one was resistant to all antibiotics .

Table4. The Number of Antibiotics (sensitive and resistant) for *K.pneumoniae* .

Isolates	Antibiotics											No. R	No. S
	GM	CIP	IPM	AMP	RIF	NIT	INN	MEM	CRO	AM	AK		
K1	R	R	S	R	R	R	S	R	R	R	R	9	2
K2	R	R	S	R	R	R	S	S	S	R	S	6	5
K3	R	R	S	R	R	R	R	R	S	R	R	9	2
K4	R	R	R	S	R	R	S	S	R	R	R	8	3
K5	R	R	S	R	S	R	S	R	S	R	R	7	4
K6	R	R	S	R	R	R	S	S	R	R	R	8	3
K7	R	S	R	R	S	R	S	S	R	R	S	6	5
K8	R	R	S	R	R	R	S	R	R	S	R	8	3
K9	R	S	S	R	R	S	R	S	R	R	S	6	4
K10	R	R	R	R	R	R	S	R	R	R	R	10	1

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