

Detection of Extended Spectrum Beta Lactamase (ESBL) in *Klebsiella pneumoniae* Isolated from Urinary Tract Infections

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الخلاصة

هدفت الدراسة الى تشخيص انتشار أنزيمات البيتا لاكتاميز واسعة الطيف في بكتريا *Klebsiella pneumoniae* المعزولة من المرضى المصابين بالتهابات الجهاز البولي الذين راجعوا مستشفى الديوانية التعليمي للفترة من كانون الأول 2013 ولغاية نيسان 2014. تم جمع 85 عينة إدرار وبعد الزرع الأولي على الأوساط الزرعية ثم الحصول على 30 عزلة من بكتريا *Klebsiella pneumoniae*. أظهرت نتائج المسح الأولي لمقاومة مضاد البيتا لاكتاميز (الامبسلين والاموكسيلين) بان جميع العزلات كانت مقاومة لهذين المضادين وبنسبة (100%).

اختبرت لاحقا مقاومة جميع العزلات لثمانية مضادات حيوية من أصناف مختلفة باستخدام طريقة انتشار القرص، وقد أظهرت النتائج ان جميع العزلات كانت مقاومة للمضادات ceftriaxone, ceftazidime, cefepime وبنسبة (100%). اما المضاد tetracycline والمضاد levofloxacin فقد قاومتها (66.6%) من العزلات كما أن (50%) من العزلات كانت مقاومة للمضادين ciprofloxacin, tobramycin وقد كانت جميع العزلات حساسة لمضاد الجنتا ميسن وبنسبة 100%.

بينت الدراسة الحالية زيادة نسبة البكتريا المنتجة لأنزيمات البيتا لاكتاميز مقارنة بالدراسات السابقة 11 عزلة وبنسبة 36.7% و المقاومة للمضادات الحياتية وخاصة الجيل الثالث والرابع من السيفالوسبورين.

Abstract

This study aimed to examine the dissemination of extended spectrum B-lactamase (ESBL) producing isolates of *Klebsiella pneumoniae* obtained from patients with urinary tract infection who admitted Al-Diwaniya Teaching Hospital from December 2013 to April 2014. A total of 85 urine samples collected, after the first culturing thirty isolates of *Klebsiella pneumoniae* were obtained. The results of primary screening showed that all isolates were found to be resistance to B-lactamase antibiotics (ampicillin and amoxicillin) at percentage (100%).

All isolates were screened for their antibiotic resistance against 8 antibiotics of different classes using Kirby-Bauer disk diffusion method. The results showed that all isolates were resistance to ceftazidime, ceftriaxone, and cefepime in (100%), while the antibiotics tetracycline and levofloxacin (66.6%) of isolates were resistance. Half of isolates were resistance against ciprofloxacin and tobramycin, all isolates were sensitive to gentamycin in percentage (100%). Present study revealed increase the percentage of ESBL producing *Klebsiella pneumoniae* 11 isolates (36.7%) in compared with other studies, also the isolates were resistance to third and fourth generation of cephalosporine.

Introduction

Antibiotic resistant bacteria cause serious nosocomial and community acquired infections that are hard to eradicate using available antibiotic. Moreover extensive use of broad-spectrum antibiotics in hospitalized patients has led to both increased carriage of *Klebsiella pneumoniae* led to and

development of multi-drug resistant strains that produce extended-spectrum beta-lactamase(ESBL). Epidemic strains of cephalosporin resistant *K.pneumoniae* have been associated with increased morbidity and mortality in hospitalized patients(1).

Klebsiella pneumoniae is among the most common gram-negative bacteria encountered by physicians worldwide. It is a common hospital-acquired pathogen causing urinary tract infection in children and adult, it can cause a classic form of primary pneumonia, enteritis, meningitis in infants and purulent abscesses(2). *Klebsiella pneumoniae* is also a potential community-acquired pathogen(3).

Urinary tract infection (UTI) is a major health problem affecting million of people each year. UTI usually starts as a bladder infection, often ascends to affect the kidneys, and ultimately, may cause renal failure, bacteremia, sever sepsis and even mortality(4).

Resistance of *K.pneumoniae* to broad-spectrum cephalosporins such as cefotaxime, ceftriaxone and ceftazidime and other B-lactam antibiotics a serious

therapeutic problem in many parts of the world (5,6). Such resistance has often been associated with transferable plasmid encoded ESBL. ESBL are more prevalent in *K.pneumoniae* than in any other enterobacterial species (7,8).

Since 1983, nosocomial outbreaks of ESBL producing *K.pneumoniae* infections in Europe and South America have been described. Between 1990 and 1992 5% of *K.pneumoniae* clinical isolates produced ESBL. In France 10 to 30% of *K.pneumoniae* strains are reported to produce plasmid mediated ESBL (9).

The epidemiology of ESBL has previously been studied and several works have describe the potential risk factors associated with ESBL *K.pneumoniae* isolates (10 ,11 , 12,13 ,14 ,15).

Materials and methods

1-Clinical specimens

Eighty five urine specimens were collected from outpatient that attended to Al-Diwaniya Teaching Hospital during the period from December 2013 to April 2014. Those patients comprised 54 females and 31 males whom aged from 19 to 71 years.

2- Bacterial isolates

Urine samples were inoculated onto Blood agar, MacConkey agar and Chrom agar. After 24 aerobic incubation at 37°C , isolates were identified to the species level using biochemical tests. Biochemical test employed were indole test, methyl red, vogas prosquar, citrate utilization, urase production, and fermentation of sugars. Sugar fermentation test performed were sucrose, glucose, manitol, lactose and adonitol. and H₂S production on Triple sugar iron. Oxidase, catalase, phenyl alanine deaminase and motility (16). Definitive identification of bacterial isolates made via vitek2-Compact System..

3- Screen for ESBL

ESBL were detected by the double disc synergy test (DDST). Synergy was determined between a disc of amoxicillin-clavulanate (20/10 μ g) and 30 μ g disc of each ceftazidime, cefotaxime, and ceftriaxone placed at distance of 30 mm. Muller-Hinton agar was used. The tested organism was considered to produce ESBL, if the zone size around the tested antibiotic disc increased towards the amoxicillin-clavulanate disc (17).

4- Antibiotic sensitivity

Antibiotic sensitivity of clinical *K.pneumoniae* isolates was done by Bauer Kirby's disc diffusion method (18). Organisms were grown on Muller-Hinton agar plates by sterile swabs then antibiotic discs were placed on media and pressed gently followed by overnight incubation. Minimum inhibitor concentration (MIC) was measured by Vitek-2.

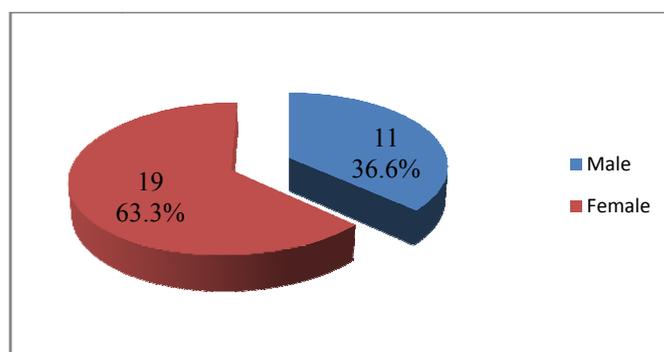
Results

A total of 30/85 (35.29%) *Klebsiella pneumoniae* isolates were obtained from patients with UTI, there were 19 (63.3%) from female and 11(36.6%) from male as shown in Figure(1).

Morphological and biochemical characterization were identified based on colonial morphology and biochemical reactions according to (16) as shown in Table (1).

A CHROM agar was used as a selective and differential media for culturing *Klebsiella pneumoniae* that was isolated from urine as shown in Figure(2)

A mong 30 isolates of *K.pneumoniae* that obtained from patients, the activities of 10 antibiotics against isolates were studied as shown in table (2). All isolates were found to be resistant to a minimum of 5 antibiotics tested. Hence they were considered to be multidrug resistant. However, the highest resistance rate were found to ampicillin, amoxicillin, ceftazidime, ceftriaxone and cefepime with 100%, tetracycline and levofloxacin 66.6%, tobramycin and ciprofloxacin with 50%.On the other hand 100% of isolates were susceptible to gentamicin, which was the most effective drug.



Figure(1) Number and percentage of *Klebsiella pneumoniae* isolated from UTI

Table (1) Biochemical tests for *Klebsiella pneumoniae*

Test	Result
Indole	-
MR	-
VP	+
Citrate	+
Urase	+
TSI	A/A
H ₂ S	-
Gas	+
Nitrate	+
Oxidase	-
Catalase	+
PAD	-
Motility	-



Figure (2) Growth of *KO pneumoniae* on A Chrom agar

Table(2) Antimicrobial susceptibility patterns and MIC of *Klebsiella pneumoniae* strains

Antibiotic	No.(%) of resistant isolate (n=30)	No.(%) of sensitive isolate (n=30)	MIC
Amoxicillin	30(100)	-	≥32
Ampicillin	30(100)	-	≥32
Ceftazidime	30(100)	-	8
Ceftriaxone	30(100)	-	16
Cefepime	30(100)	-	2
Gentamicin	-	30(100)	≤1
Tobramycin	15(50)	15(50)	8
Tetracycline	20(66.6)	10(34.4)	≥16
Ciprofloxacin	15(50)	15(50)	≥4
Levofloxacin	20(66.6)	10(34.4)	4

Discussion

Extended-spectrum beta-lactamase(ESBL) have been found in many pathogenic gram-negative bacteria, but they are most common in nosocomial isolates of *K.pneumoniae*(19), giving a proportion of 75% of ESBL-producing strains(20). ESBL were first recognized in a single strain of *K.pneumoniae* isolated in Germany(21). The genes encoding for the production of ESBLs and resistance to penicillin, ampicillin, amoxicillin, tetracycline, rifampin, and erythromycin were located on conjugative plasmids whereas genes encoding for resistance to cephalothin, cefazolin, cephalexin and gentamycin were located on the chromosome.

Many studies(22,23)have shown that *K.pneumoniae* ESBL is important nosocomial pathogen which cause bloodstream infections, urinary tract infections, respiratory infections, surgical sites infections, and skin infections.

Klebsiella pneumoniae is one of normal micro flora of intestine, it pose important virulence factors (as capsule) helping in increase the opportunity to infect urinary system. The capsule protects the bacteria from harsh conditions and increase their resistance to immune system by phagocytosis process (24). The most predictable and primary etiological bacteria involved in UTI are *Escherichia coli* followed by *K.pneumoniae* in both out and inpatient

(25). The prevalence of UTI was more in female than in male secondary to shorter urethra, closer proximity to the perirectal area in females .Out of the30 isolates of *K.pneumoniae*, 19(63.3%) were from females while 11(36.6%) were from males. UTI are more frequent in females than males (26).

Antibiotic resistance is a major clinical problem in treating infections caused by *Klebsiella* spp. The resistance to the antimicrobials has increased over the years and vary from country to country (27, 28).

Our result showed high rates of resistance in *K.pneumoniae* to B-lactam antibiotics (ampicillin and amoxicillin) in 100%. This is in agreement with other studies (29, 30). While was higher than those obtained in Hilla/ Iraq which found that 7308% of *klebsiella* isolates obtained from clinical and environmental samples(31), other recorded that 78.6% were resistant to those antibiotics (32). In Najaf/ Iraq the rate of resistance were 88% (33), and in Nigeria 66.7% were resist these antibiotics (34) while it equal to those reported in Tenzania 100% (35).

The mechanism of resistance to B-lactam antibiotics was mainly to production of B-lactamase. The high ratio of resistance was not only attributable to the production of B-lactamases. The other mechanisms conferring resistance to these compounds is caused by reducing of the activity of

B-lactam antibiotics in a resistant cell due to many factors such as, the sensitivity of the antibiotics to B-lactamase, the penetration through the outer membrane, the affinity for the target (PBPs), and the amount of B-lactamase (36).

One of the most striking findings in present study was the high level of resistance to third generation cephalosporins. All of the isolates (100%) were resistance to ceftazidime and ceftriaxone and resist to fourth generation cephalosporins cefepime in

percentage (100%). Ceftazidime resistance are marker for the presence of extended-spectrum B-lactamase (37).

Aminoglycoside have good activity against clinically important gram negative bacilli (38). Gentamycin showed good activity with 100% isolates were susceptible because it rarely used in UTI.

The observed resistance in *Klebsiella* to ciprofloxacin was 50%, this is lower than studies conducted in India(39) and higher than those of USA (40).

References

- 1-Dean A. D.; Dean, A. J.; Burton, A. H.; and Dicker, R. C.(1990).Epi-Infoversion 5:a word processing database and statistics programme for epidemiology on microcomputers. VSD. Inc Stone Mountain Ga.
- 2- Paterson, D. L.; and Sagnimeni, A. J. (2002). Community acquired *Klebsiella pneumoniae* bacteremia: global differences in clinical patterns. *Emerg Infect Dis*; 8:160-166.
- 3-Sarathbabu, R.; Ramani, T.V. ;Bhaskara rao, K. and Supriya Panda. (2012). Antibiotic susceptibility pattern of *Klebsiella pneumoniae* isolated from sputum, urine and pus samples. *Jou. of Pha. And Bio. Sci.*:4-9.
- 4- Foxman B.; Zhang L.; Tallman P.; Palin K.; Rode C.; Bloch C.; Gillespie B.; and Marrs CF.(1995). Virulence characteristics of *Escherichia coli* causing first urinary tract infection predict risk of second infection. *J Infect Dis*;172:1536-41.
- 5- Ben-Ami, R. ; Schwaber, M. J.; and Navon-Venezia, S., (2006). Influx of extended spectrum beta-lactamase-producing *Enterobacteriaceae* into the hospital. *Clin. Infect. Dis.* 42: 925- 934.
- 6- Canton, R.; Novais, A.; Valverde, A.; Machado, E.; Peixe, L.; Baquero, F.; and Coque, T. M.(2008). Prevalence and spread of extended-spectrum β -lactamase-producing *Enterobacteriaceae* in Europe. *Clin. Microbiol. Infect.*14(Suppl.1):144-153.
- 7- Winokur, P. L.; Canton, R.; Casellas, J. M.and; Legakis, N. (2001). Variations in the prevalence of strains expressing an extended spectrum β -lactamase phenotype and characterization of isolates from Europe, the Americas, and the Western Pacific region. *Clin. Infect. Dis.* 32: Suppl., S94-103.
- 8- Franiczek, R.; Krzyzanowska, B.; Dolna, I.; Mokracka, G.; and Szufarowski, K. (2005). Extended-spectrum β -lactamase-conferring transferable resistance to different antimicrobial agents in *Enterobacteriaceae* isolated from bloodstream infections. *Folia Micrbiol.* 50:119-124.
- 9-Arlet, G.; Rouveau, M.; Casin, I.; Bouvet, P. J. M.; and Philippon, A. (1994). Molecularepidemiology of *Klebsiella pneumoniae* strains that produce SHV-4-B-lactamase and which were isolated in 14 french hospitals. *J. Clin. Microbiol.*32:2553-2558.
- 10-Al-Jubouri, S. S. (1997). Genetical and molecular study of β -lactamase produced by local isolates of gram-negative bacteria. Ph.D. thesis. College of Science. Al-Mustansiriyah University
- 11-Al-Charrakh, A. H.; Al-Muhana, A.M.; and Al-Saadi, Z.H. (2000). Bacterial profile of blood stream infections in children less than three years old. *J. Babylon University.*, 6 (4):
- 12- Al-Moaiad, A.Q.(2003). Prevalence of Resistance to some β -Lactam Antibiotics among some Gram Negative Bacteria in Urinary tract Patients with Cancer. M.Sc. Thesis. College Of Science. Al-Mustansiriyyah University2.
- 13-Al-Charrakh. A. H., (2005). Bacteriological and genetic study on extended-spectrum β -lactamases and bacteriocins of *Klebsiella* isolated from Hilla city. Ph.D. Thesis. College of Science Baghdad University.
- 14-Al-Muhannak, F. H. N. (2010). Spread of Some Extended Spectrum Beta-Lactamases in Clinical Isolates of Gram Negative Bacilli in Najaf. M.Sc. Thesis. College of Medicine. Kufa University.
- 15-Al-Fatlawi, A. F. (2012). Detection of some extended spectrum beta-oli and *Klebsiella* spp. Isolated from colon and bladder cancer patients in Al-Diwaniya city.M.Sc. Thesis. College of Medicine. Al-Qadisiya University.
- 16- MacFaddin, J.F.(2000). Biochemical tests for identification of medical bacteria. Lippincott Williams & Wilkins. Philadelphia, USA.
- 17- National committee for Clinical Laboratory Standards. (2003).Performance standards for

- antimicrobial susceptibility tests, 8th ed. 45:493-496.
- 18- Bauer, A. W.; Kirby, W. M. M.; Sherris, J. C.; and Turck, M. (1966). Antibiotic susceptibility testing by a standardized single disc method. *Amer. J. Clin. Path.*
- 19- Medeiros A.A. (1993). Nosocomial outbreaks of multiresistant bacteria extended-spectrum beta-lactamases have arrived in North America. *Ann. Int. Med.* 119: 428-430.
- 20-Sirot D. (1995). Extended-spectrum plasmid-mediated β -lactamases. *J. Antimicrob. Chemother.* 36: 19-34.
- 21-Knothe H.; Shah P.; Krcmery V.; Antal M.; and Mitsuhashi S. (1983). Transferable resistance to cefotaxime, cefoxitin, cefamandole and cefuroxime in clinical isolates of *Klebsiella pneumoniae* and *Serratia marcescens*. *Infection*, 11: 315-317.
- 22-Mehrgan, H.; Rahbar, M.; and Arab-Halvahi, Z. (2010). High prevalence of extended-spectrum beta-lactamase producing *Klebsiella pneumoniae* in tertiary care hospital in Tehran, Iran. *J. Infect. Dev. Ctries.* 4(3): 132-138.
- 23-Khorshidi, A.; Rohani, M. ;and Moniri, R.(2011). The prevalence and molecular characterization of extended-spectrum-producing *Klebsiella pneumonia* isolates recovered from Kashan hospital university, Iran. *Jundishapur J. Microbiol.* 4(4):289-294.
- 24-Podschun, R.; Sievers D.; Fischer A. and Ullmann U. (1993). Serotypes, hemagglutinins, siderophore synthesis, and serum resistance of *Klebsiella* isolates causing human urinary tract infections. *J. Infect. Dis.*, 168: 1415-1421.
- 25-Gupta, K.; Hooton, T.M.; Wobbe, C. L.; and Stamm, W. E. (1999). The prevalence of antimicrobial resistance among uropathogens causing uncomplicated cystitis in young women. *Int. J. of Anti. Age.* 11(3-4):305-308.
- 27- Ibeawuchi R.; and Mbata, T. I. (2002). Rational and irrational use of antibiotics. *Afr. Health*, 24(2): 16-18.
- 28- Tonic, M.; and Goic-Barisic, I.(2005). Prevalence and antimicrobial resistance of extended-spectrum beta-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* strains isolated in a university hospital in Split, Croatia. *Int. Microbiol.* 8(2): 119-124.
- 29-Kahan, N. R.; Chinitz, D. P.; Waitman, D. A.; Dushnitzky, D. ;Khan, E.; and Shapiro, M. (2006). Empiric treatment of uncomplicated urinary tract infection with fluoroquinolones in older women in Israel: another lost treatment option. *Ann. Pharmacother.*, 40(12):2223-2227.
- 30- Aktas, E.; Yigit, N. (2002). Detection of antimicrobial resistance and extended β -spectrum beta-lactamase production in *Klebsiella pneumoniae* strains from infected neonates. *J. Int. Med. Res.* 30(4):445-448.
- 31-Orrett, F. A. (2005). Clinical sources and prevalence of resistance to antimicrobials of *Klebsiella pneumoniae* strains in Trinidad. *Saudi Med. J.* 26(11): 1766-1770.
- 32- Hadi, Z.J. 2008. Detection of Extended-Spectrum Beta-lactamases of *Escherichia coli* and *Klebsiella spp.* isolated from patients with significant Bacteriuria in Najaf. M.Sc. Thesis. College of Medicine. Kufa University.
- 33- Almohana, A. M.; Hadi, Z. J.; and Abdul-Hadi, S. (2010). Extended spectrum β -lactamase (ESBL) mediated resistance to third generation cephalosporins in urinary tract infection isolates of *Klebsiella pneumonia*. *Q.M. J.* vol.6 No. 9:142-158.
- 34- Aiyegoro, O. A.; Igbiosa, O. O.; Ogunmwoyi, I. N.; Odjadjare, E. E.; Igbiosa, O. E.; and Okoh, A. I. (2007). Incidence of urinary tract infection among children and adolescent in Ile-Ife, Nigeria. *African J. Microbiol. Res.* pp. 013-019.
- 35-Blomberg, B.; Manji, K. P.; Urassa, W.K.; Tamim, B. S.; Mwakagile, D. S.; Jureen, R.; Msanji, V.; Tellevik, M. G.; and Petersen, M. (2007). Antimicrobial resistance predicts death in Tanzanian children with bloodstream infections: a prospective cohort study. *BMC Infect. Dis.* 7: 1-14. 17.
- 36- Dowson, C. G.; and Coffy, T. J. (2000). β -lactam resistance mediated by changes in penicillin-binding proteins. In: Woodford N. and Johnson A. P.(eds). *Molecular bacteriology: protocols and clinical applications.* 15: 537-553.
- 37-Al-Charrakh, A. H.; Yousif, S. Y.; Al-Janabi, H. S. (2011). Occurrence and detection of extended-spectrum β -lactamases in *Klebsiella* isolates in Hilla, Iraq. *Afr. J. of Bio.* 10(4):657-665.
- 38- Gonzales, L. S.; and Spencer, J. P. (1998). Aminoglycosides: a practical review. *Am. Fam. Physician.* 58(8):1811-1820.
- 39- Singh, N. P.; and Goyal, R.. (2003). Changing trends in bacteriology of burns in the burn unit, Delhi, India. *Burns.* 29(2):129-132.
- 40- Fedler, K. A.; and Biedenbach, D.J. (2006). Assessment of pathogen frequency and resistance patterns among pediatric patient isolates: report from the 2004 SENTRY Antimicrobial Surveillance Program on 3 continents. *Dign. Microbio. Infect. Dis.* 56(4): 427-436.