Alnakhla
Journal of Science (AJOS)
www.alnakhlajos.com
Print ISSN: 3007 – 0198
Online ISSN: 3007 – 0201

High prevalence of OXA-1 β- lactamase genes among carbapenem resistance *Klebsiella pneumoniae* pathogens in Al-Hilla hospitals

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Received: May 13th 2025
Accepted June 15th 2025
Published: August 19th 2025
DOI:
https://doi.org/10.63799/AJOS/14.2.71

ABSTRACT

This study examined the prevalence of *bla*$_{OXA-1}$ in carbapenem resistant clinical isolates of *Klebsiella pneumoniae*. The period extended between April to August, 2011. A total of 801 samples were obtained from patients hospitalized / or attended different hospitals in Hilla city. Of these,117 isolates were specified as *K. pneumoniae*. High prevalence of *K. pneumoniae* isolates were observed in stool samples 38 (27%) followed by sputum 19 (15%). All 117 *K. pneumoniae* isolates were primarily investigated for β-lactams resistance, 91 (78%) were found to be screen positive. Beta-lactam resistance isolates were submitted to antimicrobial susceptibility testing towards 26 antibiotics by Kirby-Bauer disk diffusion method. High levels of resistance were recorded for penicillin antibiotics (carbenicillin and ampicillin) with rates of resistance (99%) and (94.5%), respectively. Seventeenth (18.7%) isolates of *K. pneumoniae* were resistant to carbapenem antibiotics. These isolates were further selected for *bla*$_{OXA-1}$ gene screening by Polymerase Chain Reaction (PCR) and confirmed in 13/17 (76.5%) of isolates.

Keywords: Carbapenem resistance, *Klebsiella pneumonia,* OXA-1 β-lactamase, extended spectrum beta lactamase, PCR.

Introduction

Klebsiella pneumoniae is a member of *Enterobacteriaceae* family, recognized over 100 years ago as a cause of community acquired pneumonia (Keynan and Rubinstein, 2007). *Klebsiella* is an important opportunistic pathogen, can cause infections of respiratory tract, nasal mucosa, pharynx and generally results in primary pneumonia, septicemia and urinary tract infection (Sikarwar and Batra, 2011). Pneumonia is the most frequent nosocomial infection (30 to 33% of cases) among combined medical- surgical intensive care units participating in the National Nosocomial Infections Surveillance System (Richards *et al*., 2000).

Resistance to β-lactam antibiotics is now a problem in patients throughout the world. The prevalence of β-lactamases among clinical isolates varies greatly worldwide and in geographic areas and are rapidly changing over time. Extended spectrum beta lactamases (ESBLs) are one type of these enzymes. The OXA – type enzymes are another growing family of ESBLs. These β- lactamases differ from the TEM and SHV enzymes in that they belong to molecular class D and functional group 2d (Bush *et al*., 1995). They are resistant to ampicillin, cephalothin, oxacillin, and cloxacillin, but are not inhibited by clavulanic acid (Thirapanmethee, 2012). Although, most ESBLs have been found in *E. coli*, *K. pneumoniae*, and other *Enterobacteriaceae*, the OXA- type ESBLs were originally discovered in *Pseudomonas aeruginosa* isolates from a single hospital in Ankara, Turkey (Hall *et al*., 1993). In this study, we aimed to detect the prevalence of *Klebsiella pneumoniae* among various clinical samples, the antibiotic susceptibility profile and the resistance gene of OXA-1 beta lactamases among carbapenem resistant isolates by Polymerase Chain Reaction (PCR) method.

Materials and Methods

Samples collection: Eight hundred and one various clinical samples (stool, sputum, vagina, burn, urine, wound, blood, ear, eye and throat) were obtained during the period extended between April to August 2011, from patients hospitalized / or attended to different hospitals in Hilla city / Babylon Province, included: Babylon Teaching Hospital for Maternity and Pediatric, AL- Hilla Teaching Hospital, Merjan Teaching Hospital and Chest Diseases Center. All samples were cultured on Blood agar, MacConkey's agar (Himedia) and incubated at 37 C˚ for 24 hrs. Isolated bacteria, *K. pneumoniae* were identified using the standard biochemical tests according to Holt *et al*. (1994); Baron and Finegold. (1994); Collee *et al.* (1996); MacFaddin (2000). Confirmatory identification was carried out by VITEK 2 system following manufacturer's instructions.

Screening Test for β -lactam Resistance: Preliminary screening of *K. pnenmoniae* isolates being resistant to β-lactam antibiotics was carried out using pick and patch method (NCCLs, 2003). Results were compared with *E. coli* ATCC 25922 (College of Medicine, University of Kufa) as a negative control.

Antimicrobial susceptibility testing: Antimicrobial susceptibility testing of β- lactam resistant *K. pneumoniae* isolates was achieved on Mueller-Hinton agar (Oxoid) plates using Kirby-Bauer disk diffusion method (Bauer *et al*.,1996). The antibiotics discs were: Ampicillin (10µg), Carbenicillin (100 µg), Piperacillin (100 µg), Amoxicillin-clavulanic acid (30 µg), Cefotaxime (30 µg), Ceftazidime (30 µg), Ceftriaxone (30 µg), Cefepime (30 µg), Cefoxitin (30 µg), Aztreonam (30 µg), Cefaclor (10µg), Cefprozil (30µg), Imipenem (10 µg), Meropenem (10µg), Ertapenam (10µg), Gentamicin (10 µg), Amikacin (30µg); Kanamycin (30µg) , Nalidixic acid (30 µg) , Ciprofloxacin (5µg) ; Levofloxacin (5µg) ; Trimethoprim- Sulfamethoxazole (25µg), Nitrofurantion (30µg), Chloramphenicol (30µg) ,Tetracycline (30µg) and Doxycycline (30µg). The cultures were incubated at 37 C° for 18 hrs under aerobic conditions and diameter of inhibition zones were measured and interpreted in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2010). *E. coli* ATCC 25922 was used as the standard strain for antibiotic susceptibility testing.

PCR detection of OXA-1 gene among carbapenem-resistant *K. pneumoniae*

Isolates: DNA from bacterial cell was extracted by salting out method in accordance with Pospiech and Neuman with some modification and used as a template for PCR reaction (Pospiech and Neuman,1995).

Detection of OXA-1gene was achieved by conventional PCR technique. The following primers (Bioneer, Korea): OXA-1/ F(5^- - ATAT CT CT ACTG TT GC ATCTCC- 3^-) and OXA-1/R (5^--AAACCCTTCAAACCATCC-3^-) were used for amplification of this gene (Karami *et al*., 2008). Amplification reaction mixture was carried out in a 25 µl reaction volume using 12.5 µl Go Taq Green Master Mix 2X (Promega), 5 µl DNA template, 2.5 µl of 10 pmol/ µl of specific up stream primers and, 2.5 µl of 10 pmol/ µl of specific downstream primers, 2.5 µl nuclease-free water. Program for PCR reaction consisted as following: Initial denaturation step at 94 C° /5min, denaturation at 94 C° /50sec, primer annealing at 55 C° /50sec, extension at 72 C° /1min and final extension step at 72 **C°** /10 min. Amplification product was run in 1.5 % agarose gels and electric current was applied at 70 volts for 2 hr. UV- Transilluminator was used to observed DNA bands, then photographed with Gel documentation system. 100 bp DNA Ladder (Bioneer, Korea) was used to assess PCR product size.

Results and Discussion

Results of the present study revealed the presence of 117 (14.6%) isolates belonged to *K. pneumonia,* table (1). This finding is in agreement with a previous local study in Hilla characterized that *K. pneumoniae* isolates comprised (15.3%) from 725 clinical samples (Hujer *et al*.,2006). Another study recorded 9.3% prevalence rate of *Klebsiella* spp. with *K. pneumoniae* being the highest recovered species (74.4) % followed by *Klebsiella oxytoca* (24.1%) Al-Saedi (2000). Same table showed that the majority of *K. pneumoniae* isolates 38/141(27%) were obtained from stool samples. High prevalence of *K. pneumoniae* in stool samples was demonstrated by other researchers, (Al-Saedi,2000) in Hilla, (14%), (Ali *et al,*2010) in Jordon, (20%). In sputum, *K. pneumoniae* was detected in 19/128 (15%) of samples. Increasing prevalence of *K. pneumoniae* in sputum was observed by other researchers, Al-Muhannak (2010), (15.7%) and Al-Sehlawi (2012), (16%).

As shown in table (2), 91/117 (78%) of *K. pneumoniae* isolates were resistant to ampicillin and amoxicillin. This result is in accordance with a study in Hilla by Al- Charrakh,2005 who stated that 73.8% *Klebsiella* isolates obtained from clinical samples were resistant to both ampicillin and amoxicillin .Higher resistant to these antibiotics could be attributed not only to the production of β-

lactamases, but also other resistance mechanisms like decrease the affinity of target PBPs or decrease permeability of the drug into the cell (Jacoby and Munoz-Price, 2005).

Table (1): Numbers and percentages of *K. pneumoniae* among different clinical samples.

Clinical sample	No. of sample	No. (%) of *K. pneumoniae* isolates
Stool	141	38 (27%)
Sputum	128	19 (15%)
Vagina	116	18 (15.5%)
Burn	153	18 (11.7%)
Urine	97	10 (10%)
Wound	60	8 (13.3%)
Blood	58	3 (5%)
Ear	30	2 (6.6%)
Eye	8	1 (12.5%)
Throat	10	0(0%)
Total	801	117(14.6%)

Table (2): β- lactam resistant *Klebsiella pneumoniae* isolates recovered from different clinical samples.

No. of *K. pneumoniae* isolates	Susceptibility to ampicillin and amoxicillin	
	No. (%) of resistant isolates	No. (%) of sensitive isolates
117	91 (78%)	26 (22%)

All 117 *K. pneumoniae* isolates were screened for their antibiotic resistance against selected antibiotic agents of different classes (Fig.1). In the present study a high resistance was observed for penicillins (carbenicillin and ampicillin) with rates of resistance 90(99%) and 86(94.5%), respectively, whereas 75(82.4%) of isolates were resistance to piperacillin. This result is in agreement with a pervious study in Hilla by Al- Asady,2009 who found that all 15 (100%) β-lactam resistant *Enterobacteriaceae* isolates were resistant to ampicillin, piperacillin and carbencillin . High resistance to this class of antibiotics may be due to widespread use of these antibiotics in Hilla hospitals. The lower resistance level was observed to carbapenem antibiotics when imipenem showed (10 %) resistance rate. In spite of the low level of resistance, this result is higher than that reported by other local studies contacted in Iraq which reported that the susceptibility of *K. pneumoniae* isolates collected from clinical and environmental samples to imipenem was (100%) (Asady, 2009; Al-Muhannak, 2010 and Al-Hilli, 2010). Other study demonstrated 2% resistance to imipenem by *K. pneumoniae* in a surveillance study in two hospitals in India (Pathak *et al*., 2012). Reasons behind resistance may be due to inappropriate duration of antibiotic therapy and subtherapeutic concentrations of the drug (Baquero *et al*., 1997 and Philippe *et al*., 1999).

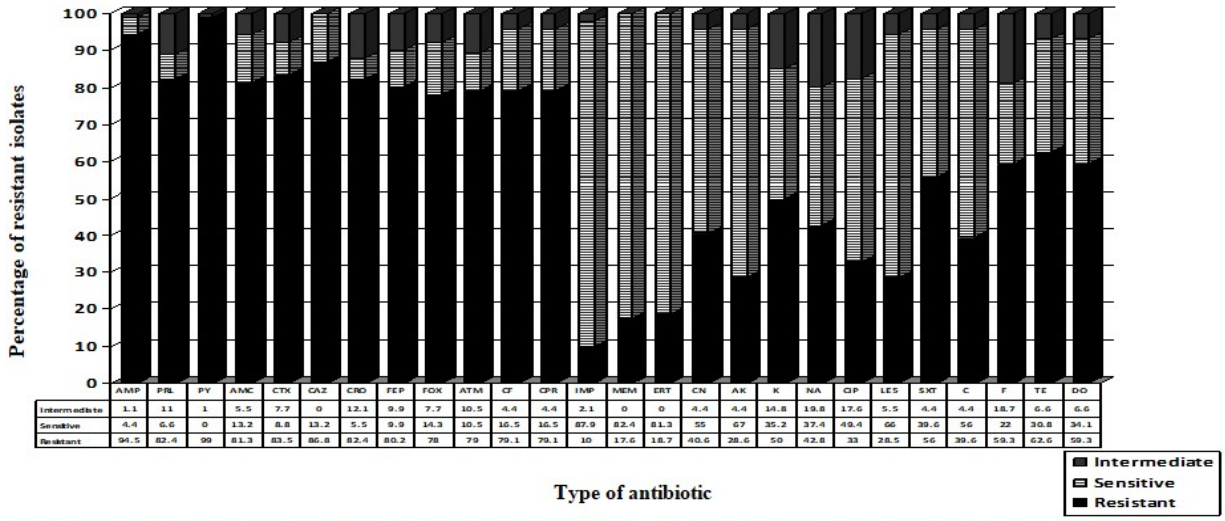

Figure (1): Antibiotics susceptibility profile of *Klebsiella pneumoniae* isolates by disk diffusion method (n=91).

AMP,Ampicillin;PRL,Piperacillin;PY,Carbenicillin;AMC,Amoxi-clav;CTX,Cefotaxime;CAZ,Ceftazidime;CRO,Ceftraiaxone;FEP Cefepime; Fox,Cefoxitin; ATM,Aztreonam; CF,Cefaclor; CPR,Cefprozil; IMP,Imipenem; MEM,Meropenem; ETP,Ertapenem; CN, Gantamicin ; AK, Amikacin ; K, Kanamycin ; NA , Nalidixic acid ; CIP, Ciprofloxacin ; LE[5],Levofloxacin ; SXT, Trimethoprim-Sulfamethoxazole;C,Chloramphenicol;F,Nitrofurantion;TE, Tetracycline;DO,Doxycycline.

Results also revealed that, 13/17 (76.5%) of carbapenemase positives *K. pneumoniae* carried *bla*$_{OXA-1}$ gene (Figure 2). OXA enzymes are regarded as OXA-type ESBls, attack the oxyimino–cephalosporins and have a high hydrolytic activity against oxacillin, methicillin and cloxacillin more than benzylpenicillin (Queenan and Bush, 2007). The most of OXA derivative genes are plasmid and integron located (Poirel *et al*., 2001). The presence of co-resistance gene cassettes on integrons make these genetic elements useful to bacteria by facilitating widespread dissemination through patients from a wide variety of clinical disciplines (Poriel *et al*., 2002). Low prevalence of *bla* $_{OXA-1}$ genes has been reported in Najaf, Al-Muhannak (2010) ,6 (9.7%) and Al-Sehlawi (2012), 2(10%) . While in Hilla city, Al-Hilli (2010) found that all *E. coli* and *Klebsiella* spp. isolates from Merjan teaching hospital were negative in OXA-PCR.

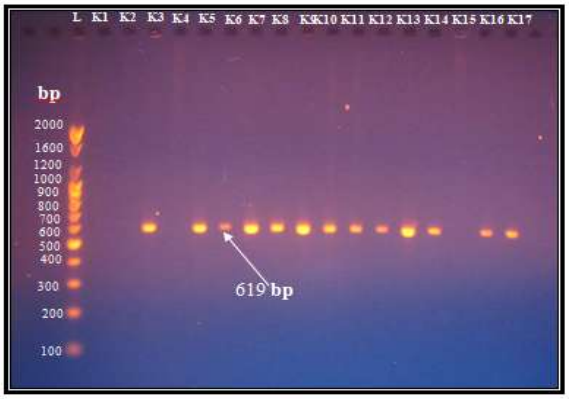

Figure (2): Agarose gel electrophoresis (1.5%agarose,70 % volt for 2-3 hrs) for *bla*$_{OXA-1}$ gene product (ampilified size 619 bp) using DNA template of carbapenem-resistant *K. pneumoniae* isolates extracted by using salting out method. Lane (L), DNA molecular size marker (100-bp ladder). Lanes (K3, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 16 and 17)of *K. pneumoniae* isolates show positive results with *bla* $_{OXA-1}$ gene. Lanes (K1, 2,4 and15) show negative results with *bla* $_{OXA-1}$ gene.

Conclusions

High prevalence of OXA-1 β- lactamase among carbapenem resistant *K. pneumoniae* isolated from patients attending Al- Hilla hospitals. The conventional PCR assay designed in this study should be applied routinely in clinical microbiology laboratories in Hilla hospitals to determine different ESBLs genes. Continued surveillance of carbapenem resistance isolates, understanding other molecular mechanisms of resistance and disseminations play a pivotal role in controlling spread and guiding antimicrobial therapy.

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