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Detection of some Resistance genes in *Klebsiella Pneumoniae* and *Proteus Mirabilis* isolated from nosocomial infection in Baghdad hospital.

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ABSTRACT

Detection of encoded genes for some of the bata-lactame enzymes of *Klebsiella pneumoniae* and *Proteus mirabilis* isolated from patients in Baghdad hospitals using morphological and molecular methods. The study included the collection of two hundred and fifty clinical samples from patients infected with Enterobacteriaceae (128 males and 122 females), the clinical sample collected from (Urine, septum, wounds, burns, pulmonary fluids, ear swabs). The samples were identified by morphological methods and chemical tests, as well as for diagnosis using the VITEK2 system. The study revealed that *Klebsiella* spp (19.2%) and *Proteus* spp (10%). Sensitivity test of isolates sample was made by using disc diffusion method. Using Polymerase Chain Reaction (PCR) and Gel electrophoreses technique with the use of specific primers based on the World Bank Gene, six pathgenic genes were detected from β-lactamas-resistant gene group (OXA-2and-20, OXA1-5and- KCP, OXA1, TEM, SHV). The results of isolating and diagnosing genes showed that *K. pneumoniae* and *P.mirabilis* had at least one of the resistance genes.

Keywords: *Klebsiella*, *Proteus, β*-lactam*,* OXA-2and-20, OXA1-5and- KCP, OXA1, TEM, SHV

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INTRODUCTION

Enterobacteriaceae is one of the most common pathogens, which are the main cause of hospital infection compared with the other Gram-negative bacteria (Mehrad et al, 2015; Huang et al, 2012).

The resistance of Enterobacteriaceae has been a global epidemiological problem in the past two decades, due to the appearance of enzymes resistant to β-lactam and carbapenem antibiotic family (Lynch et al., 2013). The problem of the production of these enzymes has been more complicated, and causing major health problems around the world. (Karlowsky *et al*, 2003; Orrett, 2004 ;Jean *et al*, 2009)

ß-Lactamase Are bacterial enzymes that disrupt the work of antibiotics through the process of hydrolysis of the lactam ring contained in the chemical composition of the antibiotic, and as a result, compounds become ineffective. There are at least 400 different species of bacteria producing ß-Lactamase enzymes isolated from clinical specimens. (Zamani *et al*, 2017) The production of ß-Lactamase enzymes is one of the most common and widespread resistance mechanisms in bacteria, where enzyme-producing bacteria are resistant to many types of antibiotics (Solano et al., 2002). It was also observed by DNA testing to detect genes which translated into the production of pathogenic enzymes to develop 775 genes related to antibiotic resistance (Frye et al., 2009).

Klebsiella and *Proteus* bacteria from the genera of Enterobacteriaceae, they cause many opportunistic diseases and many different infections to individuals, especially hospital patients and may cause death sometimes.
(Podschun and Ullmann, 1998; O'Hara *et al*, 2000; Jacobsen *et al*, 2008)

Klebsiella bacteria are clinically important, as they are resistant to treatment because they have different resistance mechanisms, especially the production of β-lactamase enzymes. (Chan et al, 2009; Saeed et al, 2009) While *Proteus* are sensitive to antibiotics unless they are gaining new resistance mechanisms (Winn et al, 2006; Wichard et al, 2005; Bononi et al, 2008)

METHODOLOGY

Sample collection: A total of 250 clinical specimens (122 females and 128 males aged from 5 to 87 years old) were collected and diagnosed from patients with Enterobacteriaceae infections of Baghdad hospitals. The study included different samples (urine 45.6% , sputum 24.4%, wounds 12.4%, burns 8.8%, Bronchi washing fluids 5.2% and ear swabs 3.6%).

Isolates identification: Bacterial isolates were identified using morphological and biochemical tests. based on what was reported in (PHE[b], 2015; Hemra *et al*, 2013; Forbes *et al*, 2007; Koneman *et al,* 2006)**.** And isolated sample were confirmatively diagnosed by VITEK2 system on central health laboratories, and based on what was reported in (Murray *et al*, 2003; Baron *et al*,1995).

Antimicrobial susceptibility testing: The isolates were screened for their antibiotic resistance against 14 antimicrobial agents of different classes using Kirby-Bauer disk diffusion method based on (Yan *et al*,2001) and interpreted according to the CLSI based on (NCCLS, 2003 and 2004; Schwalbe *et al*, 2007).

Extraction and purification of DNA: DNA was extracted and purified using Wizard®, a genome DNA purification kit (Promega, Madison, WI, USA).

Determination of resistance genes using PCR technique: To detect these genes, a specific series of target genes is amplified using PCR technique. *Klebsiella* and *Proteus* resistance genes are investigated. These genes symbolize bacterial resistance to many of the antibiotics β-lactam and carbapenem.

Preparation of primers for each gene: prepared of primers based on what was reported in (Al-Azawi, 2013, Al-Jailawi *et al*, 2014).

Polymerase chain reaction (PCR): Polymerase chain reaction is used to amplify bacterial DNA. A PCR reaction was performed using 20 µl of the reaction mixture, based on (Alfa DNA) company. After mixing the materials,

they were placed in the center of the central instantaneous output, where the interaction program was set (Al-Jailawi*et al*, 2014).

DNA fixation and gel electrophoresis: The samples were carefully placed in the holes assigned to them in the gel. The electric power was powered by 7 volts for one hour. As the nucleic acid moves from the negative pole towards the positive electrode, the dye's arrival can be seen where the gene is located. DNA packets were clearly seen under ultraviolet radiation along 365 nm, where they were filmed using a digital camera. (Magdeldin, 2012)

Statistical analysis: The results were analyzed statistically by Chisquare (SPSS -17) test at the level of significant when P-value ≤ 0.05

RESULT AND DISCUSSION

The number of Enterobacteriaceae isolated from different infections was calculated according to the age and sex groups. The total isolates which isolated from males were 128 samples and 51.2% of the total samples, while the total number of isolates which isolated from females was 122 samples and 48.8%. The results showed that the isolation of Enterobacteriaceae was in higher numbers and proportions in persons aged 21 to 60 years compared with young ages 1-20 and over 60 years of age. The results of the statistical analysis using chi square that there is no significant difference (P> 0.05), suggesting a lack of correlation between the patient's age and the number or Enterobacteriaceae ratios, as statistical analysis results suggest using the test T that there is no significant difference (P> 0.05) between The sex of the patient and the numbers or ratios of isolated bacteria.

The number and percantage of bacteria isolated from different pathological specimens, which included urine samples (114 samples and by 45.6%), samples of sputum (61 sample by 24.4%), wounds swabs (31 sample 12.4%), burns swabs (22 sample 8.8%), bronchi fluid sample (13 samples and 5.2%) and ear swabs (9 samples and 3.6%). The results of the present study indicate that the pathogenic bacteria isolated from the urin samples formed the highest percentage, which was (45.6%). The results of the statistical analysis using chi square and the T test indicated that there was no significant difference between the patient's sex and the numbers or isolates of the intestinal bacteria within each sample of the samples isolated from the bacteria, except samples taken from ear swabs. These results are consistent with the results of the study (رؤوف وآخرون، 2013)on different patient samples that were isolated from patients, where they had the highest rate of bacterial infection (50.7%). The results of the study were also agreed with the results of the study (Alshammari and Al-Skhattat, 2015) in the hospitals of Najaf Al-Ashraf governorate, where 54.08 infected bacteria were isolated from urin samples.

The results of the present study indicate that isolated Enterobacteriaceae showed a 19.2% of all samples are *Klebsiella spp.* and collected highly percentage from sputum sample (47.54%). And 10% of all samples are *proteus spp.*and collected highly percentage from burns sample (22.73%).

Enterobacteriaceae species were identified according to the phenotypic characteristics of colonies on the culture media. (Mac Faddin, 1985; Winn *et al*, 2006; Forbes *et al*, 2007)

The genotypes of Enterobacteriaceae growing on the neutrient agar were *Klebsiella* characterized by bacteria with large colonies of viscous texture, which is due to the production of the capsule in bacteria, while the *proteus* was characterized by small colonies, and the bacteria is characterized by the phenomenon of swarmming.(Hawley and Ruebush, 2017 Carroll et al., 2016). The species of Enterobacteriaceae growing on the Macconkey agar were characterized by different appearance of colony color, shape on the dish. *Klebsiella* was characterized by large, sticky, convex and pink colonies because of its ability to ferment lactose sugar in the media, which changes the color of the media from red to yellow (Koneman et al., 1992). *Proteus* colonies were characterized by their pale, semi-transparent and small size, as well as the phenomenon of swarming on the surface of the dish (Leboffe and Pierce 2011).

Some biochemical tests were conducted for the diagnosis of Enterobacteriaceae in general, where the test results indicated that all species of Enterobacteriaceae were positive for Urease and Catalase test and negative examination for Indole test. (Hemra *et al*, 2013; Koneman *et al,* 2006)

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The antibiotic susceptibility pattern:

In this study, all the *Klebsiella* spp. (n=48) and *Proteus* spp.(n=25) isolates were screened for their antibiotic resistance against 14 antimicrobial agents of different classes using Kirby-Bauer disk diffusion method .A strain is considered amultidrug resistant (MDR) if an isolate is resistant to representatives of three or more classes of antibiotics which measurement based on (NCCLS, 2003; 2004; Schwalbe *et al*, 2007)

Table 1: The antimicrobial susceptibility of *Klebsiella* spp. and *Proteus* spp. Isolates

Type of antibiotic	No.(%) of Resistant *proteus* spp.(n=25)	No.(%) of Resistant *Klebsiella* spp.(n=48)
Ciprofloxacin	2 (8%)	11 (23%)
Imepenime	4 (16%)	2 (4.2%)
Cefotaxime	18 (72%)	20 (41.7%)
Doxycycline	5 (20%)	10 (20.8%)
Gentamicin	20 (80%)	14 (29.2%)
Ampicillin	25 (100%)	48 (100%)
Azithromycin	15 (60%)	10 (20.8%)
Piperacillin	6 (24%)	25 (52.1%)
Tobramicin	11 (44%)	19 (39.6%)
Ciftrixone	5 (20%)	20 (41.7%)
Cefepime	10 (40%)	46 (95.8%)
Amikacin	_ (0%)	6 (12.5%)
Augmintin	_ (0%)	3 (6.2%)
Levofloxacin	3 (12%)	5 (10.4%)

The results indicated that *Klebsiella* was resistant against antibiotics. it showed high resistance against the antibiotics Ampicillin, Cefepime and Piperacillin with ratio 100%, 95.8% and 52.1%, respectively. While the

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resistance against both Ceftriaxone and Cefotaxime antibiotics was 41.7%, On the other hand *Proteus* showed highly resistance against Ampicillin and Gentamicin with ratio 100% and 80% respectively, more are its showed resistance against Cefotaxime with ratio 72% and against Azithromycin , Tobramycin and Cefepime 60%, 44% and 40% respectively.

The Most resistant bacteria from *Klebsiella* spp. and *Proteus* spp. obtained and they investigated by using the VITEC2 system ,thr results show that the all choose *Klebsiella* spp are *Klebsiella Pneumonia* and all *Proteus* spp are *Proteus mirabilis.*

Molecular Diagnosis for pathgenic genes

Using Polymerase Chain Reaction (PCR) and Gel electrophoreses technique with the use of specific primers based on the World Bank Gene and (Jones *et al*, 2009) , six pathgenic genes were detected from β-lactamas-resistant gene group (20 OXA-2and- OXA1-5and- KCP, OXA1, TEM, SHV). As on below table:

Table 2: pathgenic genes for *K. pneumonia* and *P.mirabilis* by using PCR and gel electrophoresis

	Most resistant Bacteria							
Pathogenic genes	K.P(1)	K.P(2)	K.P(3)	K.P(4)	P.M(1)	P.M(2)	P.M(3)	P.M(4)
OXA-1	■	■	■	■	■			■
OXA2-20				■	■	■		
OXA5-10					■	■		
TEM	■	■	■	■	■		■	■
SHV	■	■	■	■	■			■
KCP1-3					■	■		■

■ The genes which are available in each sample.

The results of isolating and diagnosing genes showed that K. pneumoniae and P.*mirabilis* had at least one of the resistance genes. The results of the genetic diagnosis using PCR teqneque are showed that the strains of the bacteria (*K. pneumoniae*), with the symbol (K.P1)(K.P2)(K.P3) carried the group genes (OXA1, TEM, and SHV), with no indication of the presence of genes (OXA2and-20, OXA-5and KCP-1to-3, OXA-10), While the Kp4 strain was the carrying the gene group (OXA2and-20, OXA1, TEM, SHV). There was no any positive result of the presence of the gene (KCP-1 to-3). These result is semi similar to (Abdulhasan *et al*, 2015; AL hamadani *et al*, 2013) which show that *K. pneumoniae* have tow gene groupe(SHV,TEM). And don't agree with (Jasim *et al*, 2017) study which show the KCP-1to-3 gene and don't agree with(Khan *et al*, 2008). Which show the KCP-2 genes.

The results of the genetic diagnosis of *P. mirabilis* bacteria (P.M1) (P.M3) (P.M4), indicated that the strain (P.M1) carrying the genes (OXA2, OXA5, KCP,OXA1,TEM,SHV), (P.M2)carrying (OXA2,OXA5,KCP) genes and (P.M3) carries only (TEM) gene, (P.M4) isolate was carried all the genes except (OXA2, OXA5).

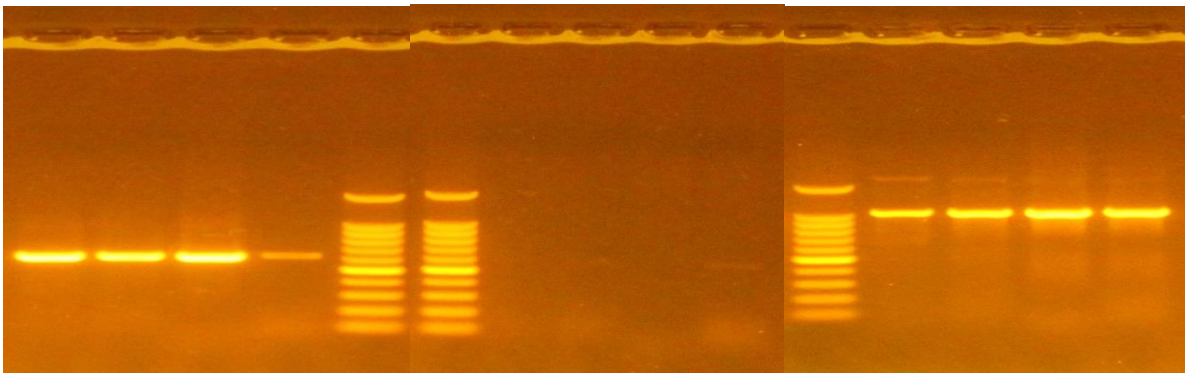

Figure 1: Ethidium bromide stained agrose gel showing PCR amplification products *K. pneumonia*.
A:OXA-1(612bp) B: OXA2and-20(524bp) C: SHV(982bp)

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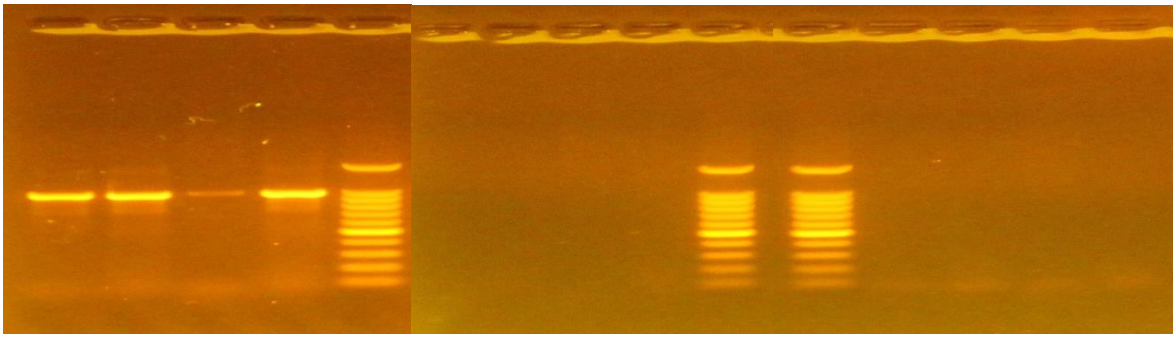

Figure 2: Ethidium bromide stained agrose gel showing PCR amplification products *K. pneumonia*. A:TEM(968bp) B: OXA5and-10(597bp) C: KCP(892bp)

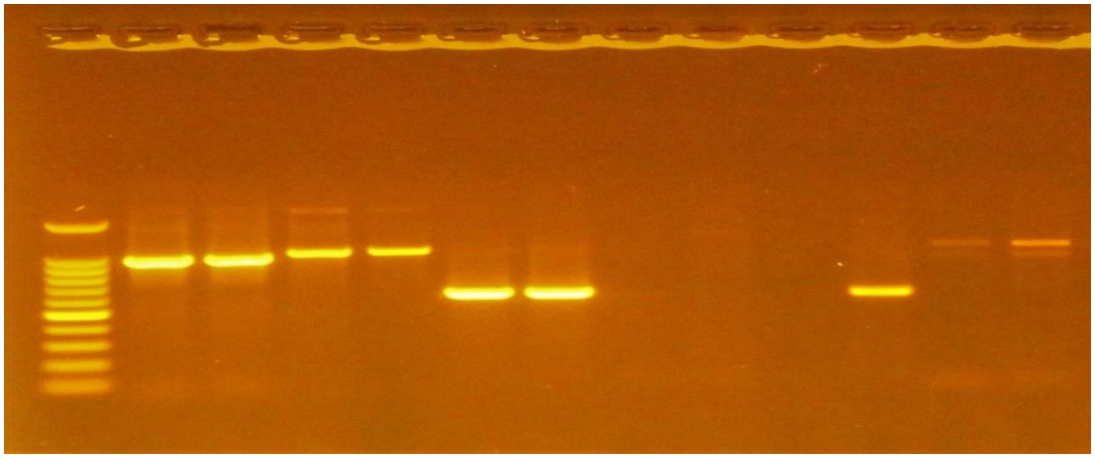

Figure 3: Ethidium bromide stained agrose gel showing PCR amplification products *P.mirabilis*.(P.M1,P.M2) for all genes.

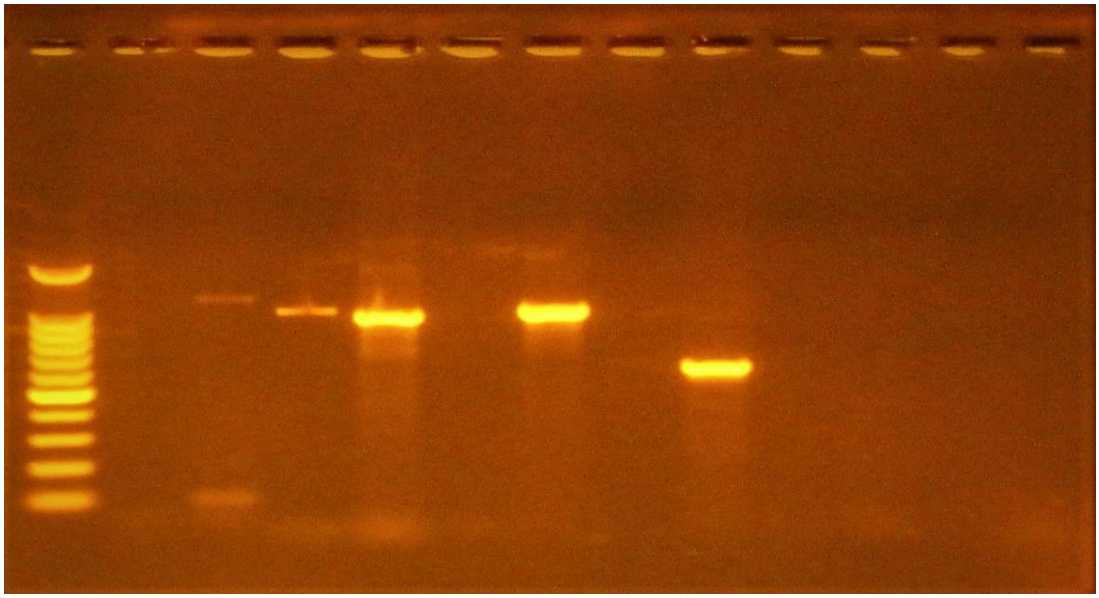

Figure 4: Ethidium bromide stained agrose gel showing PCR amplification products *P.mirabilis*.(P.M3,P.M4) for all genes.

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