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CARBAPENEM RESISTANCE-DETERMINING GENES AMONG MULTI-DRUG RESISTANT BACTERIAL ISOLATED FROM CLINICAL SAMPLES IN BASRAH GOVERNORATE

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ABSTRACT
Increasing resistance to carbapenems, may significantly reduce the choice of effective antibiotics. This study was conducted to determine the occurrence of carbapenmase producing bacteria isolates obtained from Basrah hospitals. Isolates were identified. And Carbapenem susceptibility was assayed by using VITEK 2 system. Phenotypic detection of carbapenemase was performed using four method Combined disc test, modified Hodg tests, RAPIDEC CARBA NP test, ChromID Carba media. Then isolates were subjected to monoplex PCR targeting blaNDM-1 and OXA-48 genes. 61(20.74%) Carbapenem Resist gram negative Bacteria isolates were recovered from clinical samples. 61 (100%) of isolate was found to be imipenem resistant. 31 (70.45%) of isolates give positive result to Combined disc test from 44 isolates which give positive rustle in PCR method whereas 35(79.54%) isolates showed positive results with modified Hodg test, 41(93.18%) of isolates give positive result to ChromIDCarba media, while 44(97.72%) of isolates give positive result to RAPIDEC CARBA NP test .PCR experiments showed 29 (47.54%)) isolates were harbored blaNDM-1gene and 28(45.90%) isolates were harbored OXA-48gene.

INTRODUCTION
Carbapenemases are a group of enzymes that are able to hydrolyze carbapenems even at low level (DeAndrade *et al.*, 2010; Rahmati *et al.*, 2013). There are two main molecular families of carbapenemases: serine carbapenemases, which is based on presence of serine in their active site and metallo-carbapenemases, which are a subgroup of metallo- β –lactamases (MBLs) having at least one zinc atom at their active site (Bahar *et al.*, 2010) Based on amino acid homology carbapenemases have been identified in each of the three Ambler molecular classification, however those of class A, B, and D have major epidemiological impact, Class A This group contains serine at their active site and are capable of hydrolyzing all β-lactams, such as aztreonam. In this group of carbapenemases, IMI, Sme, SFC-1 and NmcA, enzymes are mostly chromosomally encoded, (Bedenic et al., 2014) Class B carbapenemases are also known as metallo- β -lactamase since they contain two zinc ions in their active site Class B MBLs are mostly VIM and IMP types, but the recently emerged NDM-type (New Delhi metallo- β -lactamases) is becoming the most threatening carbapenemase (Levy Hara *et al.*, 2013) Class DWhich named OXAs for oxacillinases, have more than 440 known variants with 232 of them showing carbapenemase activity and majority of them are OXA-48 (Djahmi et al., 2014).

METHOD AND MATERIAL
Isolation and Identification of Isolates
One thousand and four hundered twenty two (1423) isolates were recovered from different clinical samples in six Hospitals in Basrah \Iraq during one year period starting from February, 2015 to February, 2016 Isolates were recovered from clinical samples after culturing on MacConkey agar and incubated for overnight at 37°C. For 24-48 h, and then identified by using of VITEK 2 Automated system using (GN) cards.

Antibiotic susceptibility testing
VITEK 2 system using (AST- GN327) was used, and the MIC values for these isolates were obtained The susceptibility of the isolates was determined against15antibioticsincluded:Priperacillin(PRL), Piperacillin/Tazobactam(TPZTZP), Ceftazidime(CAZ)Cefepime, (FEP)Aztreonam(ATM), imipenem(IMP), Meropenem(MEM)0, Amikacin(AK)Gentamicin(CN), Netilmicin(NTE), Tobramycin(TOP), Ciprofloxacin(CIP), Levofloxa(LVE), tetracycline(TE)Trimethoprim/ Sulfamethoxazoleremaining(SXT)

Combined disc test
Screening for MBLs was performed using disks containing 1900 µg of EDTA plus 10 µg of imipenem

disk were placed on the inoculated plates containing Muller Hinton agar. An increase of ≥ 17 mm in zone diameter in the presence 1900 µg of EDTA compared to imipenem alone indicated the presence of an MBL (Lee *et al*., 2003).

Modified Hodge test (MHT)

This test was carried out to detect carbapenemase using imipenem or meropenem as described by Clinical and Laboratory Standards Institute (2014).

RAPIDEC CARBA NP test

Carba NP is a phenotypic test that detects carbapenemases by measuring the in vitro hydrolysis of imipenem by a bacterial extract. Imipenem hydrolysis changes the pH and produces a resultant color change of a pH indicator.

Chrom ID Carba media

The used ChromID Carba medium for investigation of the producing of Carbapenemase enzymes.

PCR amplification

DNA was extracted from the isolates by using plasmid extraction mini kit according to the manufacture instructions (Bioneer Company, Korea). To amplify the genes encoding carbapenemases, a monoplex-PCR was run using the primers of NDM-1 (621bp: F/'5-GGTTTGGCGATCTGGTTTTC -3'and R/"5-CGGAATGGCTCATCACGATC -3') were designed in this study and OXA-48 gene (238 bp: F/ "5GCTTGATCGCCCTCG ATT-3' and R/"5-GATTTGCTCCGTGGCCGAAA -3') Amplification was performed in a 20µl volume as recommended by Promega Master mix instruction. PCR amplifications were carried out on a thermal cycler (Claver.England). The cycling conditions for amplification were as follows: for *bla*NDM-1 gene, initial Denaturation at 94 °C for 3 min, followed by 35 cycles of 94°C for 45s, 48°C for 35 sec and 72 °C for 35 sec with final incubation at 72°C for 7 min, and for OXA-48 initial Denaturation at 94°C for 3 min, followed by 35 cycles of 94 °C for 45s, 57°C for 35 sec and 72°C for 35 sec with final incubation at 72°C for 7 min. Amplified products were detected by agarose gel electrophoresis in 1% Tris-borate-EDTA (TBE) agarose (Promega, USA) and staining with ethidium bromide. The electrophoresis result was detected by using gel documentation system (Claver, England).

DNA Sequencing

DNA sequencing method was performed for genotyping and phylogenetic analysis study of bacteria isolates. The sequencing of the PCR product 621bp, and 238bp, for NDM-1, and OXA-48, gene respectively, the PCR product was purified by using specialized Kit.

RESULTS

One thousand and four hundered twenty two (1423) isolates were recovered from different clinical samples in six Hospitals in Basrah \Iraq during one year period starting from February, 2015 to February, 2016. Two hundered and ninety four Isolates were gram negative (294) and only sixty one (61) isolates identified as Carbapenem resistant gram negative. Which resist at less of one of antibiotic (Meropenem or Imipenem) table 1.

Table: 1. Total number of isolates.

Total	Gram Negative Bacteria	Carbapenem Resist Bacteria.
1423	294	61
100%	20.66%	20.74%

Distribution of Carbapenem Resistant Gram Negative Bacteria

Out of the 61 isolates that were found to be resistant to carbapenem,18 isolates were *Escherichia coli* (29.50%),14 *Klebsiella pneumoniae* (22.95%), seven *Pseudomonas aeruginosa* (11.47%), six isolates(9.83%) for both *Proteus mirabilis*, *Acinetobacter baumanii* , *Burkholderia cepacia* and one isolate (1.63%) for both, *Citrobacter frundill*, *Enterobacter cloacae* , *Serratia fonticola* , *Serratia marcescens* table 2.

Table: 2. Distribution of Carbapenem Resistant Gram Negative Bacteria

Total CRGNB	*E.coli*	*K. pneumoniae*	*P. aeruginosa*	*Proteus mirabilis*	*Acinetobacter baumanii*	*Burkholderia cepacia*	*Citrobacter frundill*	*Enterobacter cloacae*	*Serratia fonticola*	*Serratia marcescens*
61	18	14	7	6	6	6	1	1	1	1
100%	29.50%	22.95%	11.47%	9.83%	9.83%	9.83%	1.63%	1.63%	1.63%	1.63%

Source of the Clinical Isolates

Sixty one clinical isolates of Carbapenem Resistant Gram Negative Bacteria (CRGNB) were collected from seven sources; Burn (19) Urine (12), Blood (8), Ear swabs (10), wound (7), tissue (4) sputum (1) table 3.

Table: 3 Source of the Clinical Isolates

sources	Burn	Urine	Ear swabs	Blood	wound	tissue	sputum
CRGNB	19	12	10	8	7	4	1
Percent %	31.14%	19.67%	16.39%	13.11%	11.47%	6.55%	1.63%

Relation between Hospital unite and Carbapenem Resistant Bacteria

Twenty four 24(39.34%) of our isolates Carbapenem Resistant Gram Negative Bacteria were collected from Burn unite, nine (14.75%) from Urology unite, nine (14.75%) from ENT unite, eight (13.11%) Surgery unite, four (6.55%) from Diabetic foot unite, three (4.91%) Medicine unite, two (3.27%) from ICU, and one (1.63) from both O.P and Chest unite table 4.

Table: 4 Relation between Hospital unite and Carbapenem Resistant Bacteria

Hospital unite	Burn unite	Urology unite	ENT unite	Surgery unite	Diabetic foot unite	Medicine unite	ICU	Chest unite	O.P
CRGNB	24	9	9	8	4	3	2	1	1
Percent %	39.34%	14.75%	14.75%	13.11%	6.55%	4.91%	3.27%	1.63%	1.63%

Antibiotic susceptibility testing

VITEK 2 system using (AST- GN327) was used, and the MIC values for these isolates were obtained the susceptibility of the isolates was determined against 15 antibiotics included: Priperacillin(PRL), Piperacillin/Tazobactam(TPZ-TZP), Ceftazidime(CAZ) Cefepime, (FEP) Aztreonam(ATM), imipenem(IMP) ,Meropenem(MEM), Amikacin(AK) Gentamicin(CN),Netilmicin(NTE),Tobramycin(TOP),Ciprofloxacin(CIP),Levofloxa(LVE),Tetracycline (TE) Trimethoprim/Sulfamethoxazoleremaining (SXT).

All isolates were resistant 61(100%) to Impinem and 46(75.40%) were resistant to Meropenem the resistant to Extended-spectrum cephalosporins group Ceftazidime (CAZ) Cefepime, (FEP) were (75.40% and 78.68%) respectively Aztreonam (ATM) are antibiotic from Monbactams group which show 75.40% resistant in our isolates, Aminoglycosides antibiotic group, Amikacin (AK), Gentamicin (CN), Netilmicin (NTE), Tobramycin (TOP), show resistant (67.21%, 81.96%, 85.24%, 88.52%) respectively Ciprofloxacin (CIP), Levofloxacin (LVE), recorded resistance; 85.24% and 88.52%, in this study, resistant rate to Tigecycline about (73.77%).and to Trimethoprim/Sulfamethoxazoleremaining(SXT) about(70.49%) table.5 figure.1.

Table 3 Antibiotic resistance rates of most commonly isolated carbapenem-resistant isolates in our study

Antibiotic	No.of Resistant	No.of Intermediate	No.of Sensitive
PRl	51(83.60%)	0(0%)	10(16.39)
TPZ.TZP	48(78.68%)	2(3.27%)	11(18.03%)
CAZ	46(75.40%)	2(3.27%)	13(21.31%)
FEP	48(78.68%)	7(11.45%)	6(9.83%)
ATM	46(75.40%)	3(4.91)	12(19.67%)-
IPM	61(100%)	0(0%)	0(0%)
MEM	46(75.40%)	4(6.55%)	11(18.03%)
AK	41(67.21%)	4(6.55%)	16(26.22%)
CN	50(81.96%)	3(4.91%)	8(13.11%)
NET	52(85.24%)	3(4.91%)	6(9.83%)
TOB	54(88.52%)	1(1.63%)	6(9.83%)
CIP	52(85.24%)	5(8.19%)	4(6.55%)
LEV	54(88.52%)	2(3.27%)	7(11.47%)
TE	45(73.77%)	5(8.19%)	3(4.91%)
SXT	43(70.49%)	0(0%)	18(29.50%)

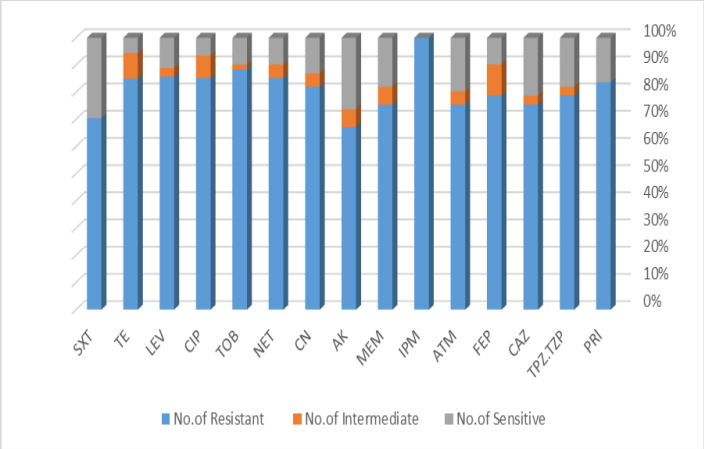

Figure.1.Antibiotic percentage resistance rates of most commonly isolated carbapenem-resistant isolates in our study

Phenotypic detection of carbapenem production

Thirty one (70.45%) of isolates give positive result to Combined disc test from 44 isolates which give positive rustle in PCR method whereas 35(79.54%) isolates showed positive results with modified Hodg test, 41(93.18%)of isolates give positive result to ChromIDCarba media, while 44(97.72%) of isolates give positive result to RAPIDEC CARBA NP test table 4.

Table 4.Phenotypic detection of carbapenem production

Combined-Diffusion -Disc Test (CDDT)	Modified Hodge test (clover leaf test) (MHT).	RAPIDEC CARBA NP test	ChromID Carba media	PCR
31	35	43	41	44
44	44	44	44	44
70.45%	79.54%	97.72%	93.18%	100%

Genotypic detection of carbapenem genes

PCR amplification of NDM -1 gene show that 29(47.54%) of isolate harbor NDM-1 gene alone or co-produce with OXA-48 gene, the high present of NDM-1 gene found in *E.coli* 14(22.95%) flow by *K.pneumonia* 8(13.11%), Pseudomonas aeruginosa 4(6.55%) Acinetobacter baumannii 3(4.91%). OXA-48 gene was present in 28(45.90%) of isolates .alone or co-produce with NDM-1, The high present of the gene was founded in K.pneumonia 13(21.31), E.coli 6(9.83%) Acinetobacter baumannii 3(4.91%), proteus mirabilis 2(3.27%), Burkholderia cepacia2 (3.27%), Enterobacter cloacae 1(1.63%), Serratia marcescens1 (1.63%). Table 5 Figure (2,3).

Table: 5. Present of genes in different isolates

Bacteria	NDM-1 gene	OXA-48 gene
Escherichia coli	14	6
Klebsiella pneumoniae	8	13
Proteus mirabilis	0	2
Pseudomonas aeruginosa	4	0
Acinetobacter baumannii	3	3
Burkholderia cepacia	0	2
Citrobacter frundill	0	0
Enterobacter cloacae	0	1
Serratia marcescens	0	1
Serratia fonticola	0	0
Total	29(47.54%)	28(45.90%)

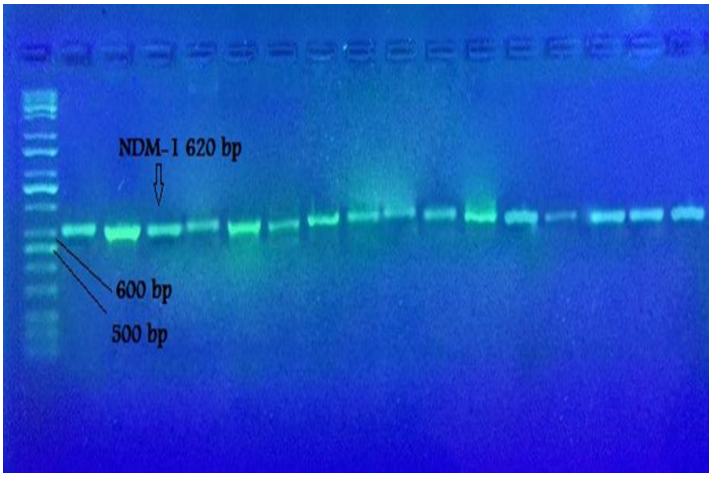

Figure 2. Agarose gel electrophoresis (1.5% agarose,70 volt for 1-2 hrs) for blaNDM-1 gene product (amplified size 620 bp) isolates show positive results with blaNDM-1 gene.

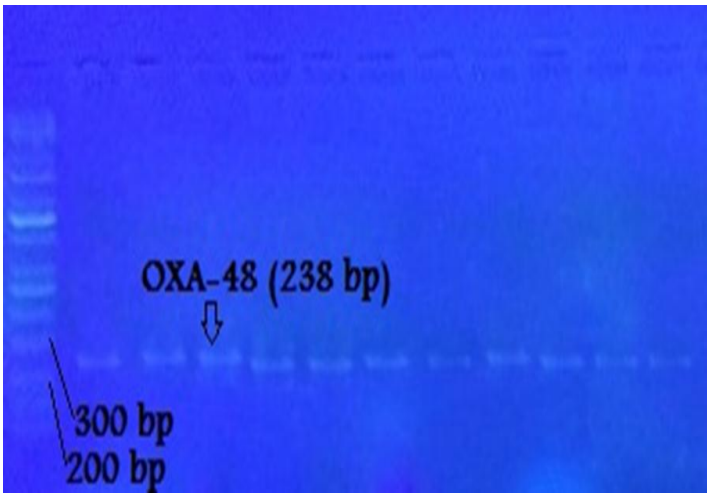

Figure 3. Agarose gel electrophoresis (1.5% agarose,70 volt for 1-2 hrs) for OXA-48 gene product (amplified size 238 bp) isolates show positive results with OXA-48 gene

Sequence analysis of NDM-1 in *E. coli*

BlaNDM-1 specific amplicons were sequenced only three isolates (E.coli 1, E.coli 11 E.coli 15) which have different pattern of antibiotic resistant. The DNA sequence identity was confirmed using BLASTN analysis. These three DNA sequences together with four similar sequences, retrieved from NCBI Gen Bank database under accession numbers (KR872634.1, KR872624.1, NC023908.1, JN255860) figure.

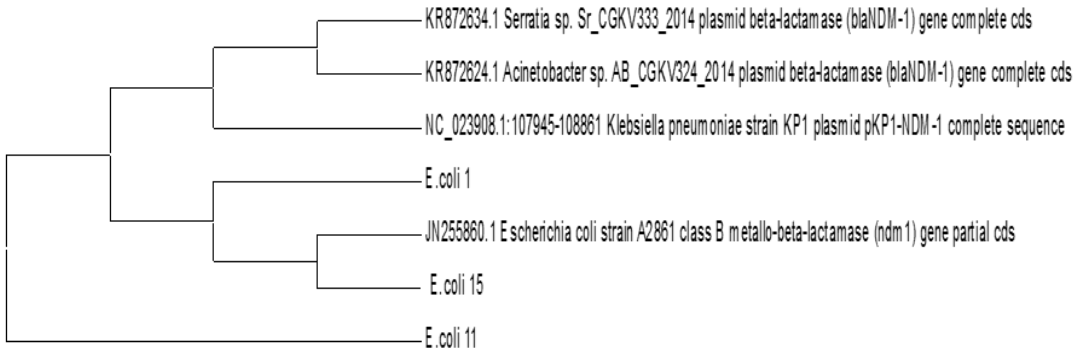

Figure 4. Phylogenetic analysis based on blaNDM-1 gene sequences obtained from the three E. coli isolates in this study and four sequences retrieved from GenBank database (NCBI).

Sequence analysis of NDM-1 in *K.pneumonia*

NDM-1 gene were sequenced two isolates (K.pneumonia.8 K.pneumonia14) which have different pattern of antibiotic resistant. The DNA sequence identity was confirmed using BLASTN analysis. These two DNA sequences together with five similar sequences, retrieved from NCBI Gen Bank database under accession numbers (LC154933.1, LC154959.1, LT615329.1, KX218441.1, KX987870.1).

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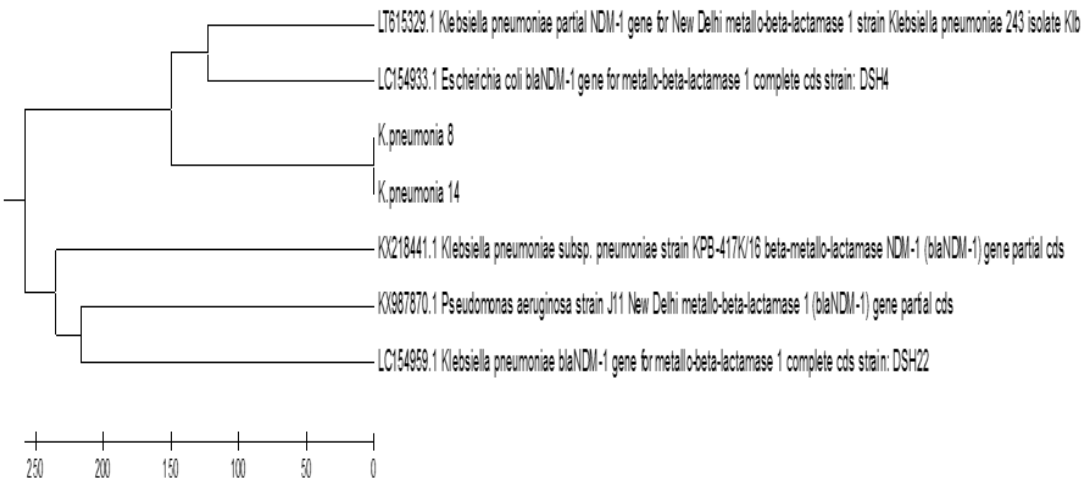

Figure 5. Phylogenetic analysis based on blaNDM-1 gene sequences obtained from the two *K.pneumonia* isolates in this study and five sequences retrieved from GenBank database (NCBI).

Sequence analysis of OXA-48 in E. coli

The DNA sequence of OXA-48gene in E. coli identity was confirmed using BLASTN analysis. The three DNA sequences together with four similar sequences, retrieved from NCBI Gen Bank database under accession numbers (KP998754.1, KM575914.1, NG_049724.1, NG_049462.1, KT175900.1).

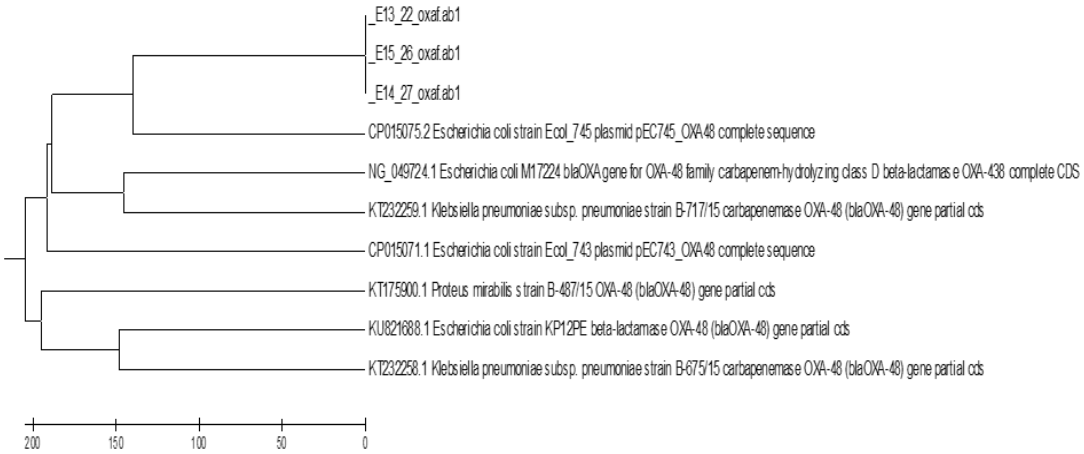

Figure 6. Phylogenetic analysis based on OXA-48 gene sequences obtained from the three E. coli isolates in this study and seven sequences retrieved from GenBank database (NCBI).

Sequence analysis of OXA-48 in *K.pneumonia*

The DNA sequence of OXA-48 gene in K.pneumonia identity was confirmed using BLASTN analysis. The three DNA sequences together with seven similar sequences, retrieved from NCBI Gen Bank database under accession numbers (KY094764.1, KY094766.1, KY094765.1,KM575914.1, KU821690.1, KU821688.1, KU821691.1).

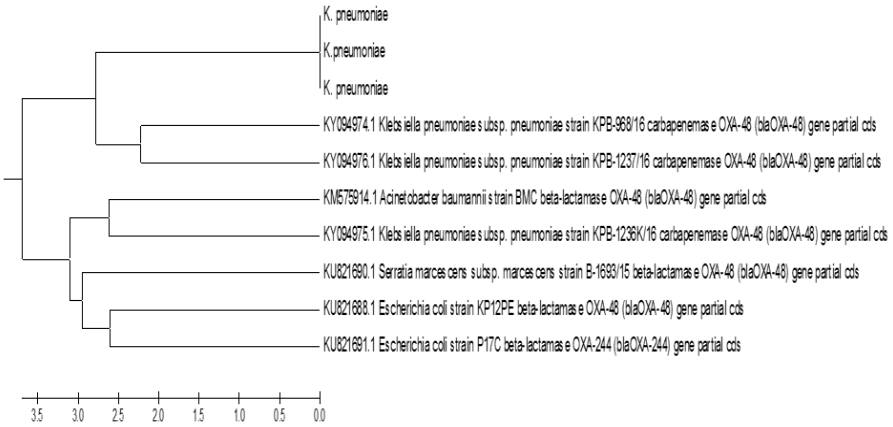

Figure: 7. Phylogenetic analysis based on OXA-48 gene sequences obtained from the three *K.pneumonia* isolates in this study and seven sequences retrieved from GenBank database (NCBI).

DISCUSSION

Carbapenem resistance in Gram-negative bacteria is increasingly encountered in healthcare-associated infections in Iraq because increasing resistance in Gram-negative bacteria has been associated with heavy antibiotic use, including carbapenems. In Iraq various studies have found different rates of carbapenem resistance In April - October 2011.a study was conducted in Sulaimani City. In this study overall Meropenem resistance was 22% From Gram Negative Bacteria (Anoar et al., 2014).in 2008 a study was conducted in Bagdad. In this study overall Imipenem resistance was 20% Pseudomonas aeruginosa (AL- Marjani et al., 2010) In the present study, the overall resistance to carbapenems was 20%, which is in comparison with the study of Manoharan et al. whose reported 17% resistance to carbapenems in Enterobacteriaceae (Manoharan et al.,2011) 15] Also, (Dutta et al.,2012; Wattal et al.,2010; and Gupta et al.,2006) showed 7.87%, 13-57% and 17-22% resistance to carbapenems respectively.

In our study high isolates were E. coli and Klebsiella pneumonia this result is in accordance with study by Chakravorty et al. in India which found that the most common Gram negative bacilli responsible for carbapenem resistance was Escherichia coli Chakravorty *et al*.2016).

According to the sources of carbepenem resistant high rate of isolates found in burns (31%) Burns provide a suitable site for bacterial multiplication and are more persistent richer sources of infection than other sources, mainly because of the larger area involved and longer duration of patient stay in the hospital (Agnihotri *et al*., 2004) .

In our study the high ratio of Carbapenem Resistant Bacteria isolate from burns unit (39%) which is alone unit in basrah and there are overcrowding in it, these led to increasing resistant bacteria to antibiotic because, Overcrowding in burns units is an important cause of cross infection which necessitates a regular monitoring of bacterial species and their antibiotic susceptibilities because significant shifts in these data may be correlated with changes in clinical management with respect to drug choice for therapy (Gupta et al., 1993).

VITEK 2 system using (AST- GN327) was used, and the MIC values for these isolates were obtained .we are use this system to determine the MIC values ,because there are many studies report the benefit of use it. Shah et al, conclude that Vitek 2 compact is an automated system and gives more accurate results than double disk diffusion method.(Shah et al.,2016) Bae IK, et al The VITEK AST- card showed high sensitivity for the detection of carbapenemases in Enterobacteriaceae strains. (Bae IK, *et al*., 2015).

All isolates were resistant 61(100%) to Impinem and 46(75.40%) were resistant to Meropenem our study show high resistant to carbapenem antibiotics compared with study in Erbil which use Vitek 2 compact system, show that in among Gram negative bacteria the most effective antibiotics that have low percentage of resistance were Meropenem (0%) and for Imipenem was 5 (6.8%) (Ahmad and Fattma, 2014) and agreement with results in study on Pseudomonas aeruginosa isolated from Public and Private Hospitals in Baghdad which show from six isolates four 66.66% are resistant to Meropenem and all of them (100%) are resistant to Impinem(Al-Charrakh et al., 2014) The isolates in our study show 83.60% and 78.68% resisted to pencilins+beta-lactamase inhibitors antibiotic group Piperacillin (PRL) and Piperacillin/tazobactam (TPZ-TZP) these results agreement with results (Hussein et al.,2013; Al-Charrakh et al.,2014).

The resistant to Extended-spectrum cephalosporins group Ceftazidime (CAZ) Cefepime, (FEP) were (75.40% and 78.68%) respectively, in other local studies, Alsehlawi, et al. (2014) and AL-kadhmi, (2015) reported that resistance rate to ceftazidime, cefotaxime and cefepime were (100%). A report by Chaiwarith, et al., (2005) documented that susceptibility to ceftazidim and Cefepime were equal, (70%) in isolates collected from Thailand hospitals. High level of resistance to third

generation cephalosporins could be attributed to the production of ESBLs, since it mediates resistance to broad spectrum cephalosporins (e.g., ceftazidime, ceftriaxone and cefotaxime) and aztreonam (Shaikh et al., 2015). Aztreonam (ATM) are antibiotic from Monbactams group which show 75.40% resistant in our isolates study which agreement with (McWilliams et al., 2014).

Aminoglycosides antibiotic group, Amikacin (AK), Gentamicin (CN), Netilmicin (NTE), Tobramycin (TOP),show resistant (67.21%,81.96%,85.24% ,88.52%) respectively Results of the present study revealed that amikacin was more effective than other aminoglycosides, gentamicin and Tobramycine. This result was parallel with other studies worldwide, as with Leepethacharat and Oberdorfer (2007) in Thailand and Özdemir, et al., (2011) In Turkey. In another study in Najaf Alsehlawi, et al. (2014) who found that resistance against aminoglycosides were (58.3%) to amikacin, whereas gentamicin and Tobramycine (83.3%). High efficiency of amikacin may be due to its less vulnerability to bacterial enzymes than other aminoglycosides. amikacin usage has been limited because prolonged use was found to cause kidney damage and injury to the auditory nerves leading to deafness (Goni-Urriza et al., 2000). The reduced use of this class of antibiotics may explain the low resistance levels of the isolates to the antibiotics in this class (Divya,2014.(gentamicin and Tobramycine resistance is often due to the expression of a variety of modifying enzymes including aminoglycoside modifying enzymes (AME), acetylases, phosphorlyases and adenylases which can impair the effectiveness of antibiotics. Other resistance mechanisms include changes in bacterial membrane permeability and altered ribosomal proteins (Barros et al., 1999).

Ciprofloxacin (CIP), Levofloxacin (LVE), recorded resistance; 85.24% and 88.52%, respectively these antibiotic belongs to Fluoroquinolones antibiotic category. Fluoroquinolones resistance results from mutations in the chromosomally encoded type II topoisomerases, and via the up regulation of efflux pumps, or point- related genes (Drlica and Zhao, 1997; Tran et al., 2005). The plasmid qnr genes play an emerging role in the dissemination of fluoroquinolone resistance (Firoozeh et al., 2014).

We found, in this study, resistant rate to Tigecycline about (73.77%). Tigecycline remains as one of the few therapeutic options for infections due to ESBL-producing isolates (Sader et al., 2013; Kelesidis et al., 2008) therefore, the usage and close monitoring of its resistance are important. (Nigo et al., 2013) Resistance to tetracycline is usually conferred through acquisition of resistance genes associated with mobile genetic elements (Roberts, 2005)the high rate of resistant to Tigecycline in our study agreement with study Taiwan, show 100% resistant to Tigecycline (Chiu et al.,2017).

The resistant rate to Trimethoprim/Sulfamethoxazoleremaining(SXT) in our study (70.49%) this result agreement with (Prakash et al.,2009; Pitout and Laupland,2008).

The high levels of resistance to antibiotics in the present study may be as a result of both intrinsic and acquired mechanisms and Carbapenem resistant bacteria mostly carry genes responsible for resistance to the antibiotics like fluoroquinolones, trimethoprimsulfamethoxazole and aminoglycosides on same transposon (Bratu et al.,2005).The resistance is widespread and constitutes serious clinical threat (Mathur et al., 2002). In addition, the selection pressure of antibiotics in hospital environment lead to multiple resistance to these drugs. El-Astal, (2005) mentioned that inappropriate and incorrect administration of antimicrobial agents and lack of appropriate infection control strategies may be the possible reasons behind increasing resistant rate of bacteria to common used antimicrobial drugs.

In our study we find 31(70.45%) of isolates give positive result to combined-disc synergy test (CDT) Different studies which have used the combined-disc synergy test (CDT) MBLs production agreement with our result , in Iraq a study witch aim determine the possibility of existence of NDM-1 gene among P. aeruginosa isolates collected from Najaf hospitals Alshara et al. found 77.8% of research isolates were positive to this test (Alshara et al. , 2014) ,other study in Irbil from 34 gram negative bacteria authors found 23 (67.6%) were positive to this test (Bakir and Fattma,2015).

Another method to detection of Carbapenemes production among the Carbapenem Resistant isolates was done by Modified Hodge test the presence of a cloverleaf shaped zone of inhibition due to carbapenemase production by the test strain was considered as positive. (Lee et al., 2001; Lee et al., 2003; Yong et al., 2002).

35(79.54%) of isolates in present study give positive results to MHT .The phenotypic assays, MHT has been suggested as the gold standard technique to detect carbapenemase producing bacteria in the past years (Nordmann et al., 2012). Our results showed that MHT failed to detect nine isolates which were PCR positive for NDM gene or OXA-48.Doyle et al., found that the sensitivity of MHT was 61% (Doyle et al., 2012). MHT is less reliable to detect NDMs, VIMs, and IMPs producing bacteria; however, it may be useful for detecting KPC and OXA-48 producers (Nordmann et al., 2011; Castanheira et al., 2011; Doyle et al., 2012).

Carba NP is a phenotypic test that detects carbapenemases by measuring the in vitro hydrolysis of imipenem by a bacterial extract. In our study the RAPIDEC CARBA NP test give high sensitivity (97.72%) of isolates which can produce carbapenemases, Different studies have reported the

Carba NP test sensitivities ranging from80% -100%, with specificity of 100%.(Nordmann et al.,2012b ; Knox et al.,2014; Vasoo et al.,2013) Our result agreement with García-Fernández et al whom, Showed that RAPIDEC CARBA NP test be fast and cost-effective, with high sensitivity (98% to 100%) and specificity (100%), (García-Fernández et al., 2016).

The current study showed that 41 (93%) isolates gave positive results on ChromIDCarba medium this agreement with result by (Vrioni *et al*., 2012; Simner *et al*., 2015).

The increasing reports on NDM-1 producing in gram negative bacteria have addressed a potential threat to global health. The high present of NDM-1 gene found in *E.coli* 14(22.95%) flow by *K.pneumonia* 8(13.11%), *Pseudomonas aeruginosa* 4(6.55%) *Acinetobacter baumannii* 3(4.91%). In Iraq NDM-1gene was report in *Klebsiella pneumoniae, Pseudomonas aeruginosa, Acinetobacter baumannii* (Poireletal2011; AL-Harmoosh and Jarallah, 2014; Anoar et al., 2014; Alshara et al., 2013) Till now there are no published works in Iraq reporting NDM-1 gene in E.coli The present study gives an initial insight on the incidence of bla NDM-1 gene in the clinical isolates of *E. coli*.

In present study we found the OXA-48 gene were increase in carbapenem resistance *Klebsiella pneumonia* isolates about (46.42%) Our result agreement with report In Moscow, Russia. During the period of January, 2013 to October, 2014, which found the blaOXA-48-carbapenemase genes were detected in 55.3 % of *K. pneumoniae*, (Fursova *et al*.,2015).

While were used to construct the phylogenetic tree in order to understand the nearest neighbor of the study sequences. The genetic divergence and homogeneity of the sequences are apparent in the phylogenetic tree Figure 5 these sequences were closely placed in the phylogenetic tree and their genetic similarity with four sequences reported from India.

CONCLUSION

Our study has shown the spreading of multidrug resistant and carbapenem resistant gram negative isolates among patients with different infections. Hence, it is suggested that, such isolates, which consequently poses an increased threat to hospitalized patients in basrah hospitals and more importantly, avoiding misuse and overuse of antibiotics may reverse the undesired effects of multidrug resistant bacteria.

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