Bacteriological Comparison Between *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* Isolated from Different Infectious Sources

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Abstract

Three hundred samples collected from different clinical sources(burns, wounds, urine,) have been isolated and identified, including 56 Isolates of *Pseudomonas aeruginosa* and 18 Isolates of *Klebsiella pneumoniae* then confirmed diagnosis by using regular api20E, api-20NE and VITEK 2 system. Results of antimicrobial susceptibility showed that all isolates were resistant to Ampicillin, Amoxicillin, Cephalexin, Nitrofurans and Co-Trimoxazole with ratio 100%, while all isolates resistant imipenem with 0%. Results of determination dominant pattern of multiple resistance to antibiotics by *Pseudomonas aeruginosa* was AM - AMX - FT- CL - SXT- Cb -CTX-CTR-PRL-ATM –CN- AK- CIP , wheals the pattern of resistance by *Klebsiella pneumoniae* was Amp –AMX- SXT-FT - CL - Cb - CTX - CTR—AK-CIP- CN - ATM. The results showed that 30 isolates of the total (56) of *P. aeruginosa* could produce β-Lactamase with percentage (53.5%), also 14(77.7%) isolates of the total (18) of *Klebsiella pneumoniae* gave positive result. *K. pneumoniae* have ability to tolerant heavy metals like (copper, zinc, mercury, cobalt) with 38.8%, 50%,16.6% , 38.8% respectively, while *P. aeruginosa* tolerant same heavy metals with percentage (17.8%, 41%, 16.6%, 25%) respectively. Additionally, results of plasmid profile presented that all isolates *of Klebsiella pneumoniae* contained tow bands of plasmid vary in size, some isolates contained the Mega Plasmid, wheals found that all isolates of *Pseudomonas aeruginosa* contained single plasmid and all sizes of plasmids were relatively close. Analysis of conjugation results showed 12(85.7%) transconjugant of *Klebsiella pneumoniae* and 25(83.3) transconjugant of *Pseudomonas aeruginosa* revised character β-Lactamase production. Results showed the highest percentage transfer of antibiotic resistance was to Ampicicillin, Amoxicillin and Carbencillin with 100%, while the lowest percentage of resistance was to Gentamicin by 35.7% in both *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. Results showed that the

highest proportion of transfer of heavy metals to zinc metal reaching to ratio 100%, whilst less transmission was mercury metal with ratio 16.6 %.

Keywords: *Pseudomonas aeruginosa*, *Klebsiella pneumonia*e, Antibiotics, Conjugation.

مقارنة بكتريولوجية بين *Pseudomonas aeruginosa* و *Klebsiella pneumoniae* المعزولة من اخماج سريرية مختلفة

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الخلاصة

جمعت ثلاثمائة عينة من اخماج سريرية مختلفة شملت(الجروح ، الحروق، الادرار) . اظهرت نتائج العزل والتشخيص وجود 56 عزلة *Pseudomonas aeruginosa* و 18 عزلة *Klebsiella pneumoniae* ، تم تأكيد التشخيص باستخدام نظام api 20E و api 20NE و نظام VITEK 2 . اظهرت نتائج الحساسية للمضادات الحيوية بان جميع العزلات كانت مقاومة لمضادات Ampicillin ، Cephalexin، Amoxicillin ، Nitrofurans ، Co-Trimoxazole وبنسبة 100% ، بينما كانت مقاومة imipenem بنسبة 0% . اوضحت نتائج النسق السائد للمقاومة المتعددة للمضادات الحيوية لبكتريا *Pseudomonas aeruginosa* بانها AM - AMX - FT- CL - SXT- Cb - CTX-CTR-PRL-ATM –CN- AK- CIP بينما لبكتريا *Klebsiella pneumoniae* كانت Amp –AMX- SXT-FT - CL - Cb - CTX - CTR—AK-CIP- CN – ATM . بينت النتائج بان 30عزلة *Pseudomonas aeruginosa*من مجموعة 56 (53.5%) كانت منتجة لانزيمات البيتالاكتاميز وكذلك 14 من اصل 18 (77.7 %) عزلة *Klebsiella pneumoniae* اعطت نتيجة ايجابية . أظهرت بكتريا *Klebsiella pneumoniae* قابلية تحمل المعادن الثقيلة (copper, zinc, mercury, cobalt) وبنسب 38.8% , 50%,16.6%, 38.8% على التوالي ، في حين تحملت *Pseudomonas aeruginosa* نفس المعادن بنسب (25%, 16.6%, 41%, 17.8%) على التوالي . اضافة الى ذلك اظهرت نتائج النسق البلازميدي احتواء *Klebsiella pneumoniae* على حزمتين بلازمية مختلفة الحجم احدهما كبيرة الحجم بينما احتوت بكتريا *Pseudomonas aeruginosa* على حزمة بلازميدية واحدة كبيرة الحجم . تحليل نتائج الاقتران الوراثي بينت 12 (85.7 %) خلية اقترانية لبكتريا *Klebsiella pneumoniae* و25 (83.3 %) خلية اقترانية لبكتريا *Pseudomonas aeruginosa* استلمت صفة انتاج انزيمات البيتالاكتاميز، وان اعلى نسبة انتقال للمضادات الحياتية هي Ampicicillin، Amoxicillin، Carbencillin وبنسبة 100% واقل نسبة انتقال لمضاد Gentamicin وبنسبة 35.7% في كلا الجنسين . بالاضاقة الى ماسبق بينت نتائج الاقتران انتقال الزنك بنسبة 100% بينما انتقل الزئبق بنسبة 16.6%.

الكلمات المفتاحية : *Pseudomonas aeruginosa* ، *Klebsiella pneumoniae*، المضادات الحياتية، الاقتران الوراثي

Introduction

Klebsiella is an important opportunistic pathogen, which is reported worldwide. It can cause infections of respiratory tract, nasal mucosa, pharynx and generally results in primary pneumonia (1). *Klebsiella pneumoniae* is a gram-negative bacteria, rod-shaped, nonmotile, facultative anaerobic, with a prominent polysaccharide capsule. This capsule encases the entire cell surface, accounts for the large appearance of the organism on gram stain, and provides resistance against many host defense mechanisms. Members of the *Klebsiella* genera typically express 2 types of antigens on their cell surface. The first is a polysaccharide (K antigen); the other is a lipopolysaccharide (O antigen) capsular. Both of these antigens contribute to pathogenicity (2). About seventy-seven 'K' antigens and nine 'O' antigens are existed. *Klebsiella pneumoniae* strains exhibit different virulence factors such as capsular polysaccharides, type 1 and type 3 adhesions, KPF-28 fimbriae, non fimbrial adhesions CF29K, factors involved in aggregative adhesions and siderophores (3). Comparative studies were carried out to detect the correlation between different capsular serotype, nonmucoid or mucoid phenotype, and the presence of different genetic structures coding for virulence factors or harbored exclusively by virulent strains (4,5). *Pseudomonas aeruginosa* is Gram-negative bacterium, frequently associated with hospital-acquired infections in intensive care units (ICUs) (6). It has been identified as the 2nd most frequent organism causing ventilator-associated pneumonia, the 4th most common causing catheter-associated urinary tract infections, the 5th cause of surgical site infections and the 7th cause of central-line-associated bloodstream infections (7). Virulence of *P. aeruginosa* is multifactorial and has been attributed to cell- associated factors like lipopolysaccharide (LPS), flagellum, alginate, pilus and non-pilus adhesions as well as with exoenzymes or secretory virulence factors like elastase, phopholipase, pyocyanin, protease, exoenzyme S, exotoxin A, hemolysins (rhamnolipids) and siderophores (8,9). In fact, coexistence of broad-spectrum β-lactamases with ESBLs, ESBLs with AmpC β-lactamase, multiple ESBLs or ESBLs with metallo-β-lactamase are common in multi-resistant *K. pneumoniae* (10). These enzymes, ESBLs are the most prevalent in *K. pneumoniae*, frequently encoded on large plasmids with size of 80–160 kb. DHA-1 is an AmpC-type β-lactamase that shares 98.7 % amino acid similarity with the

chromosomal AmpC enzyme from *Morganella morganii* and was originally described by (11) on a complex *sulI*-type integron. This integron are responsible for the transfer of bla_{DHA-1} and the corresponding *ampR* gene from the chromosome of *M. morganii* to *Salmonella enteritidis* (11). Plasmid transfer is considered as an important factor in the adaptation of microbial communities to environmental changes and in bacterial evolution (12). The transfer of plasmids has been demonstrated in situations in which it confers a selective advantage (e.g., the transfer of plasmids that carry catabolic genes in pollutant-laden environments (3, 14) and of plasmids that carry metal resistance genes in metal-stressed environments but also when there is apparently no selective advantage (15). This research aim to compare between *K. pneumoniae* and *P. aeruginosa* including antimicrobial susceptibility test, tolerance of heavy metals and possession of virulence factors and the possibility of transferring these characters.

Materials and Methods

Samples collection, Isolation and Identification

Three hundred samples collected from burns, wounds and urine, for the period 1-10-2010 to16-1-2011. Initial identification of strains of *P. aeruginosa* and *K. pneumoniae* were done on the *Pseudomonas* agar, MacConkey agar and blood agar (Biolife, Italiana). Biochemical identification of isolates was carried out by different biochemical test and conferred the diagnosis by using regular api20E, api-20NE and VITEK 2 system.

Antimicrobial susceptibility test

Fifteen antibiotics including β-Lactam group, quinolones group and aminoglycoside group have been tested in order to test the sensitivity of *P.aeruginosa* and *K. pneumoniae* by using the Muller Hinton agar plates. The incubation of strains was done at the temperature 37°C (16). The interpretation of inhibition zones around the disc is according to the guidelines of the National Committee for Clinical Laboratory standers (17). The inhibition zones were controlled with the reference *Escherichia coli* ATCC10536 and *Pseudomonas aeruginosa* ATCC154427.

Detection of β-lactamase, ESBL and Metalloβ-Lactamase Production

The acidimetric method for detection of β-lactamases was described by (WHO) 1978 (18). Disk Approximation method (19) was performed for detection of ESBLs in isolates.

Disks containing 30 µg Cefotaxime, Ceftazidime, Ceftriaxone, and Aztreonam were placed 15 mm (edge to edge) from a disk of Augmentin (20 µg Amoxicillin plus 10 µg Clavulanate) and then incubated for 16-20 hrs at 35 °C. Any enhancement of the zone of inhibition between a β-lactam disks and Augmentin disk gave an indication that the test isolate contains ESBL whose activity is inhibited by Clavulanic acid. All bacterial isolates which were positive to β-lactamase production are tested for their ability to produce ESBL enzymes. To detection Metallo β-Lactamase (MBL), method of (Bhalerao) is used, (20), using two Imipenem (10 mg) with 3 cm between them, and then the 10ug EDTA solution to one of the drives of Imipenem, incubated at a temperature 37°C for (18- 24) hr, after observing areas of inhibition zone, increase of inhibition the zone above 7 mm on the disk Imipenem with EDTA compared with the Imipenem disk alone, the result is positive .

Determination of MICs of ESBL-Producing Isolates

the two- fold agar dilution susceptibility method was used for determination of MICs of a number of different antibiotics ,the ranges of appropriate dilutions of antibiotic for MIC determination were used as(0.5 -1034) µg/ml (17).The MIC values were compared with the break points recommended by CLSI (21) .

Tolerance of heavy metals

The two- fold agar dilution susceptibility method is used for determination of tolerance isolates of heavy metals salts including (copper, cobalt, zinc, mercury). Different concentrations of salts of heavy metals ranged between 0.005 mM to 4.5 mM of each metal was carried out according to (22).

Plasmid profile (Plasmid DNA analysis) and Conjugation process

Plasmid DNA of the one isolate has been extracted by using the Pure Yield™ Plasmid Miniprep Kit (Promega U.S.A). Conjugation process was conducted in solid agar depending on the method mentioned by (23, 24). Plasmid content of the conjugate cells was done to learn transfer plasmids from the donor cells to the recipient cells, also the production β-lactamase enzyme by conjugate cells. Tests simultaneous transport of antibiotics and heavy metals was tested.

Results and Discussions

Isolation and Identification of *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*

Fifty sex isolates of *P. aeruginosa* were obtained and 18 isolates of *K. pneumoniae* of the total 300 clinical specimens (urine wounds and burns). Diagnosis was confirmed by using regular api20E, api-20NE and VITEK 2 system. Classified all isolates of *P. aeruginosa* by positions of infection (Table 1) , results inducted that the largest proportion of the isolates were within the urine samples 31(55.3%) isolates of the total, while the proportion of isolates in the wounds samples 14 (25.25 %) isolate, in cases of burns samples were 11 (19.6%) isolates

Classified all isolates of *K. pneumoniae* isolation by positions of infection, results inducted that the largest proportion 5(27.7%) isolates from urine ,wheals the proportion of isolates in the samples wounds and burns were 3,10 (16.6%,55.5%) isolates respectively (Table 1).

These results are consistent with Olayinka *et al*. (25) pointed out that the highest rate of infection with *P. aeruginosa* were from infections at the urinary tract as it amounted to (47) isolated (51.1%), followed by cases of infections of wounds and burns, which amounted to (38) case (41.3%). The results of burns with *K. pneumonia*e were comparable to the results of a local study modern conducted in Sulaimaniya hospital for burns conducted by Qader and Muhammad (26) as the percentage of *K .pneumoniae* (15%). These bacteria considered second largest causative of inflammation of burns after the *Pseudomonas* spp.

Table (1): The number of isolates and ratios as sites of infection.

Type of clinical sources	Number of Isolates	Percentage (%) of isolates
Pseudomonas aeruginosa		
Urine	31	55.3
Wounds	14	25.25
Burns	11	19.6
Klebsiella pneumoniae		
Urine	5	27.7
Wounds	3	16.6
Burns	10	55.5

Antimicrobial susceptibility test and profile of multiple resistances

Sensitivity of *P. aeruginosa* against (15) antibiotic are tested, which common types in use in our country for the treatment of various infection, the results was done by measuring the diameter of inhibition zone. These results compare with results stated in (17).The results showed a clear divergence in resistance to *P. aeruginosa* and *K. pneumoniae* to antibiotics, all isolates has resistance to antibiotics Ampicillin, Amoxicillin, Cephalexin, Nitrofurans, Carbencillin and Co-Trimoxazole with ratio 100% (Table 2), and these results are consistent with AL-Saffar (27) and Abdullah *et al*. (28) pointed out that *P. aeruginosa* were resistant to these antibiotics with 100%,. *K. pneumoniae* showed resistance to Ampicillin, Amoxicillin, Piperacillin, Nitrofurans and Co-Trimoxazole with ratio (100%), this percentage agreed with the results of Sikarwar and Batra (29) demonstrated all isolates of *K .pneumoniae* resistant to carbencillin by 100%. The results (Table 2) showed that the percentage of isolates resistant to Carbencillin amounted to 94.44%. This resistance may due to change in permeability of the outer membrane, as well as the secretion of β-lactamase enzymes and efflux pump system (30.) (Table 2) also indicates that the percentage of resistance to Cefotaxime, Ceftriaxone and Cephalexin, reached 85.71%, 85.71% and 100% respectively.

These results approach with Qasim (31) as the ratio of resistance to Cefotaxime in its Isolates 92%, and these results differ with Al-Gherawi (32) found the percentage of resistance Cefotaxime 66.7% .The results showed that *K .pneumoniae* resistant Ceftriaxone and Cefotaxime with (72.22 % and 83.33) respectively. These results agreed with Sikarwar and Batra (29) found *K. pneumonia*e resistant to Cefotaxime with ratio 76%. The difference in resistance may be return to counter this widespread use of this antibiotic. Results showed that the percentage of isolates of *P. aeruginosa* resistant to Piperacillin is 73%, and this result is agreed with Abdullah *et al.* (28) demonstrated the ratio of resistance in this amounted to 24%. The percentage of *K. pneumonia*e resistant to Aztreonam was 48.21%, while resistance *of P. aeruginosa* (33.33%), this ratio do not agree with the study carried out by Sarojamma and Ramakrishna (33) said isolates resistant to Aztreonam was 70%. The results in (Table 2) showed all isolates for tow genes sensitivity to imipenem with 100%. These results agreed with the study conducted by Ghafourian *et al*. (34) proved that all isolates *K. pneumonia*e

producing enzyme ESBLs and non-productive for this enzyme sensitive to Imipenem 100%. The occurrence of changes in the channel protein of outer membrane, including the loss of OMPK36 gives *K. pneumoniae* resistance to this antibiotic (35). The Imipenem which belongs to a group β-lactam is resistant to most enzymes β-lactamase, but crashing renal assay dehydropeptidase when given alone, so it given compound with Cilastatin. The Aminoglycoside group, which included Gentamycin and Amikacin, results indicated that resistance by *P. aeruginosa* was 60.71%, and 33.33%, respectively; Al-Gherawi (32) showed that the percentage of resistance by *P. aeruginosa* to Gentamycin is 66%, these a result approach with our study . *K. pneumoniae* was resistant Amikacin and Gentamycin with percentage 83.33% and 66.66%, respectively. Total resistance to Gentamycin of K. *pneumoniae* is consistent with Sarojamma and Ramakrishna (33) proved the resistance of K. *pneumoniae* to Gentamycin with ratio 60%. Quinolones group, which includes Ciprofloxacin and Ofloxacin, *P. aeruginosa* has resistance against tow antibiotics 23.21% and 5.35% respectively. While isolates of *K. pneumoniae* resistant Ciprofloxacin by 72.22%, wheals gave resistant to Ofloxacin 5.55% .This percentage agreed Yedekci *et al*.(36) said the resistance of *K.pneumoniae* with ratio 66.6%. Results of determination dominant pattern of multiple resistance to antibiotics by *P. aeruginosa* showed that the profile of resistance was AM - AMX - FT- CL - SXT- Cb -CTX-CTR-PRL-ATM –CN- AK- CIP , while the pattern of resistance by *K.pneumoniae* was Amp –AMX- SXT-FT - CL - Cb - CTX - CTR—AK-CIP-CN - ATM.

The resistant of antimicrobial quinolones by *K.pneumoniae* by mediated efflux pumps that cause multiple resistance to antibiotics which organized by mediated 38 genes , these genes are directly responsible for the development of resistance to antibiotics such as quinolones, through a change in the molecule target and reduce the permeability (36).This result disagree with Abdullah *et al*. (28) pointed to the proportion of Ciprofloxacin resistance is 4%, also found that all isolates were sensitive to Ofloxacin.

Table (2) the percentage of resistant to different antibiotics by *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*.

Antibiotics	Resistance of *P. aeruginosa* (%)	Resistance of *K.pneumoniae* (%)
Ampicillin	100	100
Amoxicillin	100	100
Carbencillin	100	94.44
Piperacillin	73.21	100
Co-Trimoxazole	100	100
Nitrofurans	100	100
Cephalexin	100	88.88
Cefotaxime	85.71	83.33
Ceftriaxone	85.71	72.22
Aztreonam	48.21	33.33
Imipenem	0	0
Amikacin	30.35	83.33
Gentamycin	60.71	66.66
Ciprofloxacin	23.21	72.22
Ofloxacin	5.35	5.55

Determine of Minimum Inhibition Concentration of a number of antibiotics

Minimum Inhibition Concentration (MIC) against (7) antibiotics , was determined because they represent antibiotics most commonly used (Drug of choice) to treat various infections caused by *P.aeruginosa* and *K.pneumoniae* in our hospitals, adopted a breakpoint (Break point) described by CLSI (21) as the basis for calculating the response is defined as optimum focus, which can is received anti-serum that provides the highest end of the treatment, is the object responsive (Susceptible) when the amounts (MIC) calculated less than stopping point.

Results indicate as shown in (Table 3) that all isolates of *P.aeruginosa* and *K.pneumoniae* resistant to Ampicillin and Amoxicillin ranged between (512-1024) µg / ml.

As for Piperacillin has been able of *P.aeruginosa* resistant concentration ranged between (256-512) µg / ml. Neu (37) pointed out that bacterial strains producing enzymes β-Lactamase type TEM able to destroy the Carbencillin as well as Piperacillin. *K. pneumoniae* is resistant to Piperacillin with rang concentration 512-1024 µg / ml. MIC of Cephalexin, Cefotaxime and Ceftriaxone value (128-1024), (16-1024), (16-1024) µg/ml, respectively by *P.aeruginosa*, while *K.pneumoniae* showed resistance to Cephalexin (64 -1024) µg/ml Cefotaxim (256-16) µg/ml, and Ceftriaxone ranged between (128-8) µg/ml. Highly resistance to cephalosporin belong to broad using of antibiotics, which results in the development of resistance, especially through the target site or by effluxes pump , this mechanism of encodes a chromosomal genes (38). MIC values for Ciprofloxacin (64-4) and (1-64) µg/ml by *K. pneumonia*and and *P.aeruginosa* respectively.

Table (3) MIC to different antibiotics by *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*.

Antibiotics	MIC(µg/ml)of *K.pneumoniae*	MIC(µg/ml)of *P.aeruginosa*
Ampicillin	512-1024	512-1024
Amoxicillin	512-1024	512-1024
Cephalexin	64-1024	128-1024
Cefotaxime	16- 256	16-1024
Ceftriaxone,	8- 128	16-1024
Piperacillin	512-1024	256-512
Ciprofloxacin	4-64	1-64

The initial selection of isolates resistant to ampicillin

The results Showed all isolates were resistant to ampicillin with concentration of 100 µg / ml, which gives an impression of lack of effectiveness of this counter against bacteria, and could have been caused bacterial resistance to this antibiotic because random use of antibiotic, this was confirmed by Sanders & Sanders(39).

Detection of β-lactamase, Extended Spectrum β-lactamase and Metallo β-lactamase Production

The results showed that 30 isolates of the total (56) of *P. aeruginosa* gave a positive result for the examination of production β-lactamase with percentage (53.5%), while the results showed that 14(77.7%) isolates of the total (18) of *Klebsiella pneumonia*e gave the result is positive (Table 4), and there is a clear difference in the time to give the result of positive ranged from several seconds to 2 minutes. β-lactamase enzyme works to reduce iodine to iodide and be the last to have lost its ability to interact with starch and the formation of a complex violet turns directly to the color white, and can be explained by the difference in time on the basis of the concentration of the enzyme β-lactamase product in periplasmic spaces , Perez and Hanson, (40) noted that reducing iodine to iodide depends on the concentration of the enzyme product in periplasmic spaces in addition to temperature and pH. Whenever the concentration of the enzyme, the more time was needed for the emergence of the positive result is less, and vice versa, and it is up to the encoded genes for these enzymes were plasmid or chromosomal (41). All isolates which producing β-lactamase subjected to detect of production ESBLs and Metalo β-Lactamase enzymes. The results indicated that 4(13.3%) isolates of the total (30) of *P. aeruginosa* were ESBL producers Table (4), the results of this study approach with Strateva *et al.* (42) pointed out that the production *of P. aeruginosa* enzymes ESBLs by (66.8%) , while the Alipour *et al.* ((43) found that (30%) of the isolates *P. aeruginosa* isolated from Iran's hospitals could producing enzymes ESBLs ; 5(35.7%) isolates 5(35.7%) isolates of the total (14) isolates were ESBLs producer, additional the results came approach with a study conducted by Shakib *et al. (*44) on *K. pneumonia*e producing these enzymes, as production rate of approximately (42.3%).

The results showed that 11(36.6%) of the total 30 produced Metalo β-Lactamase, while 8 (57.1%) isolates of *K. pneumonia*e has produced this enzyme (Table 4), and this percentage came close with Charan *et al*.(45) as the percentage of production *K .pneumonia*e for this enzyme from 71.9 - 50% and also this ratio close relative with a study by Patel *et al*. (46) as the percentage of the production of bacteria for this enzyme is 40% and this means that this enzyme makes bacteria resistant to a wide range of β-lactam by making antibiotics ineffective (47) .

Table (4). The percentages of isolates producing β-lactamase enzymes

P. aeruginosa		*K. pneumoniae*	
β-lactamase production	Ratio %	β-lactamase production	Ratio %
β-lactamase	53.5	β-lactamase	77.7
ESBLs	13.3	ESBLs	35.7
Metaloβ-Lactamase	36.6	Metaloβ-Lactamase	57.1

Tolerances of isolates some heavy metals

The results shown in Table 5 indicate that (38.8 %) of *K. pneumoniae* has ability to tolerances 1.5 mM of cobalt, while 25% of *P. aeruginosa* tolerant this same concentration. As for copper, the highest concentration was 3 mM, isolates *K. pneumoniae* tolerant this metal by (38.8%) , whereas tolerance *P. aeruginosa* this concentration by 17.8%. Copper is associated with particular sites on any amino acid for microorganism and restores oxidation-reduction cycle and generates free radical hydroxide near the binding sites of this causing damage to amino acid (48). Able 3 (16.6 %) isolates of *K. pneumoniae* tolerant a higher concentration of 0.03 mM of mercury , and *P. aeruginosa* tolerant same concentration with percentage reached to 30.3% .The mercury resistance accomplish mediated detoxification enzyme in both gram negative and positive bacteria, there is a group of genes encode for the production of periplasmic spaces associated with membrane protein. The periplasmic spaces collect Hg^{+2} from the environmental surrounding and then transmitted to the cytoplasm to treat with process of oxidation and reduction (49**).** The results suggest that (50 %) *K. pneumonia* has ability to tolerant zinc metal with concentration 1.5 mM (Table 5),
while 41.% of *P. aeruginosa* tolerant same concentration . The resistance of bacteria to heavy metals may be due to the presence of a conjugative plasmid that mediates resistance to metals, in some nosocomail isolates of the Enterobacteriaceae have been recorded had plasmids encode resistance to various heavy metals (50) .

The relationship between the isolates were resistant to antibiotics and heavy metals

Isolates under study showed different responses to antibiotics and heavy metals ranged between resistance and sensitive to antibiotics. Isolates of K. *pneumoniae* isolated from the urine has ability to resistant 15 (93.8%) antibiotic and tolerance four heavy metals (Zn, Hg, Cu, Co) by (100%), while anther isolates isolated from burns of resistance 6 antibiotics with (37.5%) and tolerance 1 (20%) heavy metal. Kumar *et al*. (51*)* pointed out that isolates of *K.pneumoniae* have multi-resistance to antibiotics that cause infections of the urinary system and epidemiological because these bacteria occur of special genes resistance to antibiotics.

Table (5) Percentages of tolerance isolates for have metals

Have metals	Concentration of have metals(Mm)	Percentage (%) of isolates
Pseudomonas aeruginosa		
cobalt	1.5	25
Copper	3	17.8
mercury	0.03	30.3
zinc	1.5	41
Klebsiella pneumoniae		
cobalt	1.5	38.8
Copper	3	38.8
mercury	0.03	16.6
zinc	1.5	50

Plasmid profile for of *P. aeruginosa* and *K. pneumoniae*

Plasmid profile of isolates has been investigated ,especially those that showed multiple resistance to antibiotics and tolerant of heavy metals, ,the results showed the all isolates *of K.pneumoniae* contained tow bands of plasmid vary in size, some isolates contained the Mega Plasmid (Fig 1). These results according with results *Wei et al*. (52) which found all

isolates *K.pneumoniae* containing two bands of plasmid different molecular weight, and also agreed with the results of Al-Charrakh *et al* . (53) pointed out that isolates of *K.pneumoniae* contain Mega Plasmid that encodes multiple antibiotic and heavy metal resistance. All isolates of *P. aeruginosa* contained one band plasmid. Most of studies indicated that the plasmids extracted from *P. aeruginosa* that carry multiple resistance to antibiotics is relatively large (54).

Conjugation

The results showed the success of the 12 process of conjugation out of all isolates of *K.pneumoniae* and *P. aeruginosa* with 100%, and there was a similarity in the pattern plasmids belonging to the original cells and transconjugant cell (Fig.1). Frifelder (55) indicated that the presence of transmitted plasmids facilitates transmission of nonconjugative plasmids which contain the encoded genes for enzymes specialized cut in one strand, called this process Mobilization, this explains the transmission of all large and small plasmids from the donor to recipient cells .

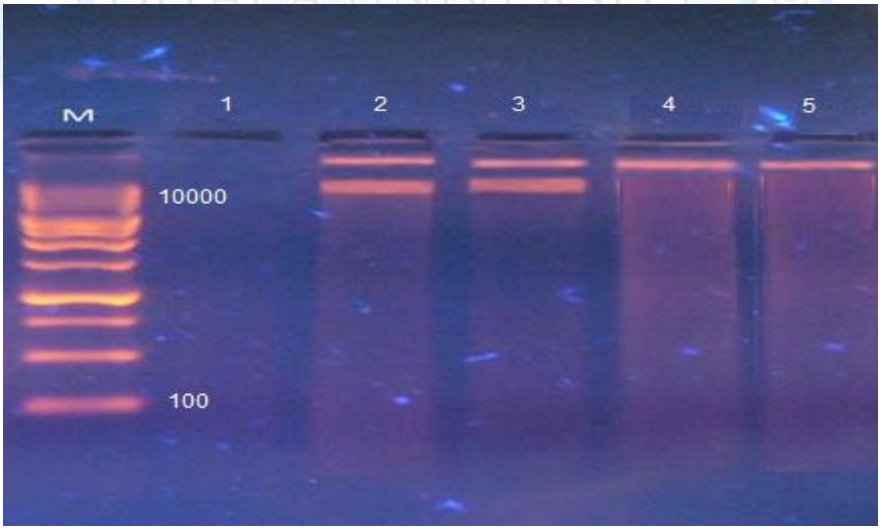

Figure (1): plasmid content of the donor cells *K.pneumoniae, P. aeruginosa* and recipient cell (*E.coli*MM294), the concentration of the agarose gel 0.7% and 7.5 volts / cm for 90 minutes .Lanes M, Leader DNA (1000 bp) ;1,content plasmid standard strain of *E.coli* MM294 ; 2, is the content of *K.pneumoniae* ; 3, plasmid content of transconjugant of *K.pneumoniae* ; 4, a plasmid content *P. aeruginosa*; 5, plasmid content of transconjugant *P. aeruginosa*.

Transfer the character β-Lactamase production, antibiotic resistance and tolerances of heavy metals.

Investigating about the transfer character for the production of the enzyme beta-Lactamase from isolates of *K.pneumoniae* and *P. aeruginosa* was studied, the results showed 12(85.7%) transconjugant of *K.pneumonia* and 25(83.3) %) transconjugant of *P. aeruginosa* revised character β-lactamase production. These results confirm that the character of the production of this enzyme portable by plasmids gene coded to production β –lactamase. These results agreed with Poirel *et al.* (*56*) pointed out that most of the enzymes beta lactamase newly discovered under the control of mega plasmids encode multidrug resistance as well as the responsibility to tolerance heavy metals. Investigate of Extended spectrum β-lactamase was performed, the results inducted that four (80%) transconjugant of *K.pneumonia*e and three (75%) transconjugant of *P. aeruginosa* were able to production this enzyme .These results suggesting that the character of the production of this enzyme may be by plasmid or may be by chromosome. Seven (87.5%) transconjugant of *K.pneumoniae* and ten (90.9%) transconjugant of *P. aeruginosa* were able to production of Metallo β -lactamase**.**

The results indicate transmission most antibiotics and heavy metals from the donor to recipient cells. Notes results from table (6) indicate the highest percentage transfer of antibiotic resistance was to Ampicicillin, Amoxicillin and Carbencillin with 100%, while the lowest percentage of resistance was to Gentamicin by 35.7% in both *K.pneumoniae* and *P. aeruginosa*. These results agreed with Al-Charrakh *et al.* (53) found the transmission character of resistance Gentamicin associated with genes located on chromosome. Results showed that the highest proportion of transfer of heavy metals to zinc reached to ratio 100%, whilst less transmission was mercury with ratio 16.6 %. These results agreed with Rouch *et al.* (57) referred to some sort of resistance to heavy metals such as mercury, are carried on chromosomal genes. Baron *et al.* (58) indicated that the genes to be located on plasmids or gene of transpose responsible for resistance of the heavy metal, and these plasmids in some cases carry genes resistant to some antibiotics Ampicicillin, Amoxicillin and Carbencillin, which have the ability to move from donor cell to recipient cells by mediated conjugation or transduction process.

Table (6) Character transmitted to transconjugant cells

Characters	transconjugant of *K.pneumoniae*	transconjugant of P. *aeruginosa*
β-Lactamase production	85.7	83.3
Extended spectrum β-Lactamase production	80	75
Metallo β –Lactamase production	87.5	90.9
ampicicillin	100	100
amoxicillin	100	100
carbencillin	100	100
gentamicin	35.7	35.7
zinc	100	100
mercury	16.6	16.6

Conclusion

The results suggest the possibility of isolating *P. aeruginosa* and *K. pneumoniae* from different clinical sources. These isolates were resistant to many antibiotics and tolerant of heavy metals. And the possibility of transmission of this antibiotics and heavy metals by conjugation process.

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