Antibiotic resistance of *Klebsiella pneumoniae* isolates from in patients with burn infections

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المستخلص:

تم جمع 210 عينة سريرية من مرضى الحروق تراوحت اعمارهم بين 5-50 سنة للفترة من اذار الى اب للعام 2016 من المرضى الراقدين في مستشفيات مدينة بغداد . اظهرت نتائج العزل البكتيري ان عدد العزلات المفردة (112) منها (42) عزلة (37.5%) تعود لبكتيريا الكليبسيلا الرئوية و 36 عزلة (32.14%) تعود لجنس الزوائف و20 عزلة (17.86%) لبكتريا اشيرشيا القولون و 10 عزلات (8.93%) لبكتريا العنقوديات الذهبية و 4 عزلات (3.57%) لجنس المثقبيات .بينما اظهر العزل المختلط تواجد الكلبسيلا الرئوية مع جنس الزوائف بنسبة 65.31% (64 عزلة) و جنس الزوائف مع الاشيرشيا القولون 18عزلة (18.37%) و الكلبسيلا الرئوية مع اشيريشيا القولو 7 عزلات (7.14%) وجنس الزوائف مع جنس المثقبيات 4 عزلات (4.08%) و الكليبسيلا الرئوية مع العنقوديات الذهبية 3 عزلات (3.06%) و جنس المثقبيات مع اشيرشيا القولون عزلتين (2.04%).

اظهرت نتائج فحص الحساسية لمقاومة بكتريا الكليبسيلا الرئوية تجاه المضادات قيد الدرسة الـ doxycycline (100%) و tetracycline (95.23%), و cefotaxime و piperacillin (85.71%) و ceftriaxone (88.09%) و trimethoprim -sulfamethoxazol (83.71%) وticarcillin (78.57%) و aztreonam (71.2%) و ceftazidime (69.04%) و ciprofloxacin (59.52%) و gentamycin (26.19%) و imipenem (21.42%) و meropenem و amikacin (19.04%) . كذلك اظهرت النتائج حساسية عالية لهذه البكتريا تجاه الـ gentamycin (78.57%) و meropenem و amkacin (76.19%) و imipenem (69.04%) .

Abstract:

A total of 210 specimens were collected from burn wound patients their ages ranged from 5-50 years during the period March to August 2016 from inpatients whom attended to hospitals in Baghdad /Iraq. The bacterial agents were showed that single pathogens growth isolates only 42 isolates (37.5 %) of *Klebsiella pneumoniae* , 36 isolates (32.14%) *Pseudomonas* spp., 20 isolates (17.86%) *Escherichia coli*, 10 isolates (8.93%) *Staphylococcus aureus* and 4 isolates (3.57%) *proteus* spp. .While mixed growth isolates frequency showed *K. pneumoniae and Pseudomonas spp.*64 isolates (65.31 %) , *Pseudomonas* spp. and *E. coli* 18 isolates (18.37%), *K. pneumoniae* and *E. coli* 7 isolates (7.14%), *Pseudomonas* spp. and *proteus* spp. 4 isolates (4.08 %) , *K. pneumonia*e and *Staph. aureu*s 3 isolates (3.06 %) and *proteus* spp. and *E. coli* 2 isolates (2.04 %). The results susceptibility testing showed that frequency resistance of *K. pneumoniae* isolates to: doxycycline were (100 %) ,tetracycline (95.23%) , cefotaxime and pipracillin (85.71%) ceftriaxone (88.09%), trimethoprim sulfamethoxazol (83.33%) , ticarcillin (78.57%), aztreonam (71.2%) , ceftazidime (69.04%) , ciprofloxacin (59.52%), gentamycin (26.19%) , imipenem (21.42%) , meropenem and amikacin(19.04 %). Also the results showed the highest frequency of susceptibility among *K. pneumonia*e was associated to gentamycin (78.57%) , meropenem and amikacin (76.19%) and imipenem (69.04%).

Key words: Burn wound, *Klebsiella pneumoniae* , Antibiotics susceptibility.

Introduction:

Burn wound infections are the most important and potentially serious impairments that occur in the acute period following injury [1]. These wounds were subsequently colonized by microorganisms, including gram-positive bacteria, gram-negative bacteria and yeasts, which derived from the host's normal flora and hospital environments [2, 3]. Also microorganisms might reach to a patient's burn lesions via contact with contaminated external environ-mental surfaces, water, fomites, air, hydrotherapy treatment, and the soiled hands of health care workers [4, 5].

Burn wound infections occurred by common pathogens such as *Pseudomonas aeruginosa, Klebsiella spp.* and *Staphylococcus aureus,* through producing a number of virulence factors that were important in the pathogenesis of invasive infections [6,7]. *Klebsiella pneumoniae,* which is opportunistic pathogens, isolated from bacteremia, pneumonia, urinary tract, soft tissue infections and burn wound infection. In addition, it is the principle cause of death in burn patients [8]. *K. pneumoniae* could easily persist in hospitals patient by hands of healthcare personnel [9], also it had become significant pathogens in nosocomial infections [10].

Using of antibiotics in different ecological roles and facilitating factors such as travel, contaminated food, poor sanitation in the community, lack of infections control in hospitals and non-antibiotic selection are the main selectors of resistant bacteria and mobile genetic elements conferring resistance [11].

The aim of this study was to isolate *K.pneumoniae* causing burn infections and to determine the prevalence of resistance among these bacteria against antibiotics which used to treat burn infections in patients referred to some teaching hospitals in Baghdad, Iraq.

Materials and Methods

Specimen collection:

A total of 210 sample swabs were collected from burn-wound patients (n=210) for the period March to August from inpatients whom attended to hospitals of Baghdad City: Al-Karama Teaching Hospital, Special Burn Hospital, Central Teaching Laboratories, Child Protection Teaching Hospital and Imam Ali Hospital. All specimens were labeled and transported by transport media (Al-Hanoof factor, Jordan)with aseptic technique to the laboratory within 1-2 hrs. Then streaked on blood agar, MacConkey agar and Eosin methylene blue agar (EMB) (Hi media /India). These swab samples were recovered from patients their age ranged from 5-50 years. After incubation for 24hrs. at 37˚ C,

the isolated pathogens were identified depending on the micro-scopic examination , morphological, biochemical tests and confirmation was done using API 20 E System [12].

Antibiotics sensitivity:

Antibiotic susceptibility test was performed by the Kirby-Bauer disc diffusion method using Muller-Hinton agar (Biomark Lab., Pune. India). The isolates were screened for suscep-tibility to the antibiotics : amikacin(30 µg), aztreonam (30 µg),cefotaxime (30µg), ceftazidime (30 µg), ceftri-axone(30 µg), ciprofloxacin (5µg), doxycycllin(30 µg), Gentamycin (10 µg), imipenem(10 µg) ,meropenem (10µg), pipracillin(100 µg), tecarcillin (75 µg) , tetracycllin (30 µg) and trimethoprim+ sulfamethoxazole (1.25 +23.75 µg). A bacterial suspension was prepared by picking up 1-2 colonies from 24 hr. cultures in to 2.5 ml of sterile distilled water. The suspension was spread on Mueller-Hinton Agar plate by sterile swabs in different directions. Anti-biotic disks were placed onto the cultures medium surface by sterile pair of forceps. The culture plates were incubated at 37°C for 24 hours; then inhibition zones around the antibiotics disks were measured [13]. The inhibition zones were controlled with the reference *E. coli* ATCC 25922 and *K. pneumoniae* ATCC 700603.

Statistical Analysis:

The Statistical Analysis System–SAS, (2012) program was used to effect of difference factors in the study parameters. Chi-square test was used to significant compare between percentage and least significant difference -LSD test was used to significant compare between means in this study.

Results and Discussion:

Isolation of bacterial agents were showed that single pathogens growth isolates only 42 isolates (37.5 %) of *Klebsiella pneumoniae*, 36 isolates (32.14%) *Pseudomonas* spp., 20 isolates (17.86%) *E. coli*, 10 isolates (8.93%) *Staphylococcus aureus* and 4 isolates (3.57%) *proteus* spp. (Table 1) .While mixed growth isolates frequency showed *K. pneumoniae and Pseudomonas spp.*64 isolates (65.31 %) , *Pseudomonas* spp. and *E. coli* 18 isolates (18.37%) , *K. pneumoniae* and *E. coli* 7 isolates (7.14%) , *Pseudo-monas* spp. and *proteus* spp. 4 isolates 4.08 % , *K. pneumonia*e and *Staph. aureu*s 3 isolates (3.06 %) and *proteus* spp. and *E. coli* 2 isolates (2.04 %) table **2**.These swab samples were recovered from patients their ages ranged from 5-50 years.

According to the (Tables **1)** and (**2**) *K. pneumoniae* were isolated in high percentages 42 isolates (37.5%) while other 70 single isolates (62.50 %) with high significant differences $P<0.01$. Previous studies indicated that *K. pneumoniae* were

preceding all nosocomial gram-negative bacteria, so they accounted in an average (15 - 42 %) among different hospitals in Iraq [14, 15, 16, and 17]. Other studies had indicated that *K. pneumonia*e as nosocomial infections were a major cause of morbidity and mortality among several burn patient inhabitants [18, 19].

Table 1: Distribution of single pathogenic agents through 112 isolates among burn patients.

Type of bacteria	Frequency	Percentage %
Klebsiella pneumonia	42	37.5
Pseudomonas spp.	36	32.14
Escherichia coli	20	17.86
Staphylococcus aureus	10	8.93
proteus spp.	4	3.57
	112	**100**

Table 2: The percentage of *K. pneumoniae* compares to pathogenic isolates.

Isolates	No. of isolates	Percentage (%)
Klebsiella pneumoniae	42	37.50
Other isolates	70	62.50
Total	112	100
Chi-square	---	9.026 **
P-valuee	---	0.00281
**** (P<0.01)**		

According to (Table **3**) *K. pneumonia*e and *Pseudomonas spp*. were the most common mixed growth 64 isolates (65.31%), *Pseudomonas spp.* and *E. coli* 18 isolates 18.37% with

Significant differences (P<0.01). The high total number of mixed growth 98 (Table **3)** might be due to many reasons like; burn wounds were suitable site for multiplication and infection of bacteria because of the large area involved and long duration of patient stay in the hospital. Further more to the contamination in the hospital environment mainly in the operating theatre, patient beds, medical instruments and hand carriers [20]. This result was inconsistent with Qader and Muhamad [16] who found that the most common mix growth isolates were *Pseudomonas spp.* and *K. pneumoniae* and agreed with him that *K. pneumoniae* were the most common single isolated pathogenic bacteria from burn wound infections**Table 3:** Distribution of mix pathogens isolated and percentage**.**

Type of mix bacteria	Frequency	Percentage%
Klebsiella pneumoniae and *Pseudomonas spp.*	64	65.31
Pseudomonas spp. and *E. coli*	18	18.37
Klebsiella pneumoniae and *E. coli*	7	7.14
Pseudomonas spp. and *proteus* spp.	4	4.08
Klebsiella pneumoniae and *Staphylococcus*	3	3.06

aureus		
proteus spp. and *E. coli*	2	2.04
	98	**100**

Results of the current study, as in (Table **4)** proved that the highest distribution of burn wound infections found within the age group 11-20 years (18 patients 42.8%) with significant differences P < 0.01. This result was in agreement with Abdul Basit *et al*.[21] who found that the burn wound infections distributed among age group 11-20 years (24 patients 48%) and it was more susceptible to burn wound infections .While Anvarinejad *et al.*[22] had found that the age group 16-36 years was more 40 patients (72.7%) susceptible to burn wound infections.

Table 4: Distribution of *K. pneumo-niae* according to the age groups.

Age groups (years)	No. of patients	Percentage for infection (%)
5 -10	2	4.8
11 -20	18	42.8
21 -30	12	28.6
31-40	3	7.1
41 -50	7	16.7
Total	**42**	**100**
Chi-square 10.184		
P-value		**0.00094**
(P <0.01)		

In the present study, results listed in (Table **5)** showed that there were 29 of females 69% and 13 of males 31% have infection and the age range from 5 to 50 year with high statistical significant difference (P<0.01) . This result inconsistent with Pondei *et al*. [23] who found that there is no significant difference between the type of organism isolated and the sex of the subjects (p = 0.66) . While Rajput, *et al*., [24] was in agreement with this study who found that burn wound infections in females (60%) was more than in males (40%) in India. Other study by Kalantar *et al*. [25] found that the burn wound infection in males was (46.5 %) and (53.5 %) in female.

Table 5: Distribution of *K. pneumonia*e according to patients.

Age(years)	N0. of patients	Female	Male
5-10	2	2	-
11-20	18	12	6
21-30	12	8	4
31-40	3	1	2
41-50	7	6	1
Total and percentage of infection	**42**	**29 69%**	**13 31%**
Chi-square (P-value)		**9.753 ** (0.00361)**	
**** (P<0.01)**			

All the 42 *Klebsiella pneumoniae* isolates were tested for their antibiotic susceptibility against 14 selected antibiotics by using Kirby-Bauer disk diffusion method to

measure the diameter of inhibition zones around antibiotic discs and compered with NCCLS[26] .The inhibition zones were controlled with the reference *Escherichia coli* ATCC 25922 and *K. pneumoniae* ATCC 700603. (Table 6) and (Figure 1**)** showes that frequency resistance of isolates. doxycycline were (100%) , tetracycline (95.23%) , cefotaxime and pipracillin (85.71%) ceftriaxone (88.09%) , trimethoprim-sulfamethoxazol (83.33%) , ticarcillin (78.57%) , aztreonam (71.42%) , ceftazidime (69.04%) , ciprofloxacin (59.52%) , gentamycin (23.80%) , imipenem (21.42%) , meropenem and amikacin (19.04 %) .

The most active antibiotics against all isolates of *K. pneumoniae* were meropenem and amikacin (76.19%) followed by gentamycin (71.42%) , imipenem (69.04%) and ciprofloxacin (38.09%) , while the minimum active antibiotic were tetracycline 2.38% followed by cefotaxime and pipracillin (9.52%) , ceftriaxone (11.90%) and ticarcillin (16.66%) . The highest frequency of susceptibility among *K. pneumoniae* isolates were associated to meropenem and amikacin (76.19%), gentamycin (71.42%), imipenem (69.04%), ciprofloxacin (38.09%), ceftazidime and aztreonam (19.04%).

In local study at Baghdad city; Al-Qafaji, [27] pointed that (100%) and (94.5%) of *K. pneumoniae* isolates were resistant to Cefotaxime and Ceftazidime, respectively. Al-Obadi, [28] referred that (90.5%), and (97.5%) of isolates were resistant to cefotaxime, and imipenem, respective-ely. The present study have percentage resistance (85.71%) and (21.42%) to cefotaxime and imipenem respectively in compared to local studies but not agree with imipenem result in local study.

Also Kevin *et al*. [29] pointed that the resistance of *K. pneumoniae* isolates to cefotaxime 69.5%, ceftazidime (68.5 %) and these results relatively in agreement with the present study. This variation in resistance might be due to the widespread use of the cefotaxime, furthermore *K. pneumoniae* isolates had a large plasmid conferred resistance to this antibiotic [30].

The isolates showed high percentage of resistance to Pipracillin antibiotic 85.71% and this result was in agreement with the study that was done by Fazeli *et al*.[31] who found that 88.4% resistance to pipracillin , while relatively in agreement with the study that was done by Al-Sa'adone,[32] who pointed that the resistance of *K. pneumoniae* isolates to pipracillin was (97.2 %). On the other hands AL-Taai, [33] referred that the resistance of K. *pneumoniae* isolates to pipracillin was (100%).

The isolates showed resistance percentage to aztreonam (71.42%). This percentage agree with the study done by Sarogamma and Ramakrishna , [34] who pointed that the

resistance percentage of *K. pneumoniae* isolates to this antibiotic was 70 % and Malini *et al.* [35] revealed (96.6%) resistance to aztreonam, while Lina, [36] pointed that all isolates were (100 %) sensitivity to aztreonam.

The isolates showed resistance percentage to trimethoprim-sulfame-thoxazol (83.33%). While studies by AL-Taai, [33] recorded that *K. pneumoniae* isolates showed resistance (100 %). Also this result was relatively agree with the study was done by Abdolaziz *et al.* [37] who found that the percentage of resistance (83%).

The isolates showed resistance percentage to Tetracycline 83.33%. This percentage was agree with the study that done by Sarogamma and Ramakrishna, [35] who documented (83%) resistance to this antibiotic, while Lina, [36], Najmadeen, [38] and Al-Qafaji, [27] showed (71.5%) , (39.5%), (32.5%) resistance respect-ively.

The isolates showed the resistance percentage to Doxycycline 100%. This percentage disagree with the study done by Mama, *et al*. [39] who found the percentage of resistance to this antibiotic (71%).

K. pneumoniae isolates showed resistance percentage to Ceftriaxone (88.09%). This percentage was consistent with the studies were done by Doumith *et al*. [40] , Al-Obadi, [28] who reported that (87%) , (87.5%) resistance respectively. While Al-Taai, [33] found the percentage of resistance to ceftriaxone (72.22%). The difference in resistance might be return to counter this widespread use of this antibiotic, change in permeability of the outer membrane, as well as the secretion of β-lactamase enzymes and efflux pump system [41].

The isolates showed the lowest resistance percentage to meropenem (19.04 %).This result was in agreement with the study done by Masoume *et al*., [42] who found that the percentage of resistance to meropenem was (20.5 %) and this finding might be due to the limited use of this antibiotic as routine antibiotic treatment.

The isolates showed the resistance percentage to (Gentamycin 23.80%). This result disagrees with the studies were done by Al-Qafaji, [27] (67.5%),

Al-Zengena, [43] (63.6 %), Masoume *et al*., [42] [45] (41.3%).While the result was relatively in agreement with the study was done by Dua'a, [44] who found that the resistance percentage to Gentamycin (16.6 %).

The isolates showed the resistance percentage to Amikacin (19.04 %). This result was inconsistent with the study was done by Malini *et al*. [46] (2014) who found that the percentage of resistance to amikacin ranges from (76.6 %) to (100 %). On the other hand, Dua'a, [44] found that the resistance percentage was (22.22 %).

The isolates showed the resistance percentage to Ciprofloxacin (59.52%). This

result was in agreement with the study done by Mshana, [45] who found that the percentage of resistance to Ciprofloxacin (54 %). While this result was relatively agree with the study done by Yedekci *et al*. [46] who found that the percentage of resistance to Ciprofloxacin was (66.6%). High percentage of resistant for these antibiotics could be attributed not only to the production of β-lactamases, but also other resistance mechanisms. Li, and Nikadio, [47] mentioned that there were three further resistance mechanisms include conformational changes in Penicillin binding proteins (PBPs), permeability changes in the outer membrane, and active efflux of the antibiotic.

The isolates showed the resistance percentage to ticarcillin 78.57%. This result was disagree with the studies done by Breurec *et al*. [48], Aljanaby and Alhasani, [49] who found that the percentage of resistance to ticarcillin was (100%).

On the other hand, the isolates showed the resistance percentage to trime-thoprim-sulfamethoxazol (83.33%).

This result was agree with the study was done by Abdolaziz *et al*. [37] who found that the percentage of resistance (83%).

Table 6 : Antibiotic susceptibility tests of isolated strains of *K. pnuemoniae*.

Antibiotics	Susceptible	Intermediate	Resistance
Amikacin	32 (76.19%)	2 (4.76%)	8 (19.04 %)
aztreonam	8 (19.04%)	4 (9.52%)	30 (71.42%)
cefotaxime	4 (9.52%)	2 (4.76%)	36 (85.71%)
ceftazidime	8 (19.04 %)	5 (11.90%)	29 (69.04%)
ceftriaxone	5 (11.90%)	-	37 (88.09%)
ciprofloxacin	16 (38.09%)	1 (2.38%)	25 (59.52%)
doxycycline	-	-	42 (100%)
gentamycin	30(71.42%)	2(4.76%)	10 (23.80%)
imipenem	29 (69.04%)	4 (9.52%)	9 (21.42%)
meropenem	32 (76.19%)	2 (4.76%)	8 (19.04 %)
pipracillin	4 (9.52%)	2 (4.76%)	36 (85.71%)
tetracycline	1 (2.38%)	1 (2.38%)	40 (95.23%)
ticarcillin	7 (16.66%)	2 (4.76%)	33 (78.57%)
trimethoprim-sulfamethoxazol	7 (16.66%)	-	35 (83.33%)

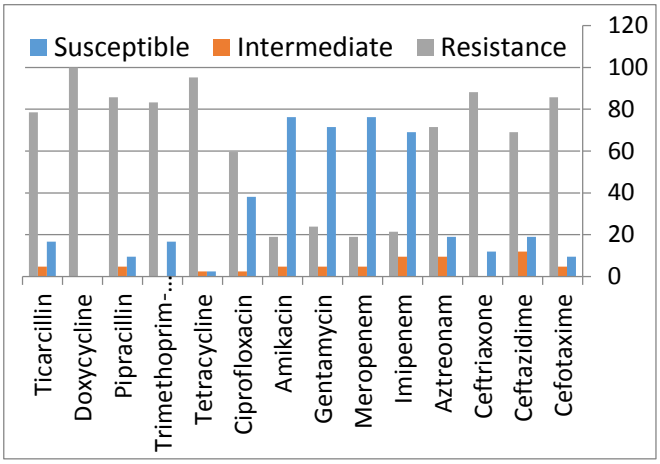

Figure 1 : The percentage of resistance of the antibiotics.

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