Detection of imipenem resistant hypervirulent *klebsiella pneumonia* in UTI patients

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Abstract

Klebsiella pneumonia is a very deleterious microbes that is essentially take gram dye staining & considered gram negative when stained by gram staining method and appear microscopically as bar or rod in shape which have very mucoid large specific colonies on blood agar medium due to potential ability to produce large glycocalyx and muco- polysaccharides that link together to secrete large biofilm and slime layer which in turn increase invasive ability of microbes to host and become resistant to several effective drugs , increase these ability being widely observed in highly evolutionary strains that have multi-virulent factors like muco-polysaccharide capsule , slime layer , biofilm in association with extremely drug resistant genes , so these bacterial strains now is demonstrated as hypervirulent *Klebsiella pneumonia* (hvKp) involved in several chronic infections worldwide .To identify the new resistant hypervirulent strains of *Klebsiella pneumonia* in hospitalized patients suffering from chronic urinary tract infections (UTI) along with catheters related patients and to determine the ability of these dangerous strains to resist strong effective antibiotics like Imipenem .Urine samples were collected from 150 urinary tract infection patients admitted in different hospitals and specialized centers of Baghdad province , then all specimens were manipulated in laboratory and cultured in special bacterial media to improve *Klebsiella pneumonia* species and to exclude other bacteria , also further identification were done by selective tests like biochemical estimation of IMVIC and vitic-2 system along with biofilm determination and antibiotic sensitivity for certain antibacterial agents.

This study have results that depend on hypervirulent ability of new emergence bacterial strains to resists the lethal action of strong bactericidal agents like imipenem, so 70% females and 30% males from 100 Klebsiella *pneumonia* isolates were 41% resistant to imipenem were as only 4% *Esherishia.coli (E. coli(* resistant strains to imipenem from a total of 150 urine samples (100 were *Klebsiella pneumonia* & 50 were *E. coli*). High percentage of imipenem resistance by *Klebsiella pneumonia* bacteria indicates that these isolates differ in virulence ability to classical nosocomial strains of *klebsiella* and have multi-resistant genes on their plasmid and chromosomal DNA that play

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essential role in wide distribution among peoples and from a medical points that become a very sever to be treated and controlled.

Keywords: *hypervirulent Klebsiella pneumonia (hvKp), Esherishia.coli (E. coli), urinary tract infections (UTIs), tryptic soy broth (TSB), phosphate buffered saline (PBS).*

اكتشاف الكلبسيلة الرئوية عالية الضراوة المقاومة للاميبينيم في مرضى التهاب المسالك البولية

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الخلاصة

Klebsiella pneumonia عبارة عن ميكروبات ضارة للغاية تأخذ أساسًا صبغة الجرام السالبة عند تصبيغها بطريقة تصبيغ الجرام وتظهر مجهريًا على شكل شريط أو قضيب يحتوي على مستعمرات كبيرة مخاطية كبيرة جدًا على وسط أجار الدم بسبب القدرة المحتملة على إنتاج جلايكوكاليكس كبير والسكريات المخاطية التي ترتبط ببعضها البعض لتفرز طبقة غشاء حيوي كبيرة وطبقة جلاتينية لزجة والتي بدورها تزيد من قدرة غزو المايكروبات للجسم وتصبح مقاومة للعديد من الأدوية الفعالة ، وتزيد من هذه القدرة التي يتم ملاحظتها على نطاق واسع في السلالات التطورية للغاية التي لها عوامل ضارة متعددة مثل الغشاء المخاطي. - كبسولة عديد السكاريد ، وطبقة الجلاتينية ، والغشاء الحيوي الرقيق بالاشتراك مع الجينات شديدة المقاومة للأدوية ، لذلك تظهر هذه السلالات البكتيرية الآن على أنها *Klebsiella pneumonia* (hvkp) المساهمة في العديد من الالتهابات المزمنة في جميع أنحاء العالم . التعرف على السلالات الجديدة شديدة الضراوة المقاومة الخاصة ببكتريا *Klebsiella pneumonia* في المرضى الراقدين في المستشفى الذين يعانون من التهابات المسالك البولية المزمنة مع المرضى ذوي الصلة بالقسطرة الأنبوبية وتحديد قدرة هذه السلالات الخطرة على مقاومة المضادات الحيوية القوية الفعالة مثل Imipenem تم جمع عينات البول من 150 مريضا مصابا بعدوى المسالك البولية في مختلف المستشفيات والمراكز المتخصصة في محافظة بغداد ، ثم تم التعامل مع جميع العينات في المختبر وزرعها في وسائط بكتيرية خاصة لتحسين أنواع *Klebsiella pneumonia* واستبعاد البكتيريا الأخرى ، وكذلك تم إجراؤها تحديد اكثر دقة لهذا النوع عن طريق اختبارات انتقائية مثل التقدير الكيميائي الحيوي لنظام imvic ونظام الـ vitic-2 مع استخدام البايوفيلم وحساسية المضادات الحيوية لبعض المواد المضادة للبكتيريا .

هذه الدراسة لها نتائج فريدة تعتمد على القدرة الفائقة لظهور سلالات بكتيرية جديدة لمقاومة التأثير المميت للعوامل القوية المضادة للجراثيم مثل Imipenem، لذا فإن 70٪ من الإناث و 30٪ من الذكور من 100 عزلة *Klebsiella pneumonia* كانت مقاومة 41٪ لمادة Imipenem في حين ان 4٪ فقط من سلالات esherishia.coli (e.coli) المقاومة Imipenem من إجمالي 150 عينة بول (100 كانت عبارة عن *Klebsiella pneumonia* و 50 عينة منe.coli) و تشير نسبة عالية من مقاومة Imipenem لبكتيريا *Klebsiella pneumonia* إلى أن هذه العزلات تختلف في القدرة على الاصابة إلى سلالات المستشفيات التقليدية من *Klebsiella pneumonia* ولديها جينات متعددة المقاومة على البلازميد و DNA الكروموسومي الذي يلعب دورًا أساسيًا في التوزيع الواسع بين الأشخاص ومن الناحية الطبية التي أصبحت شائعة جدا ويجب معالجتها والسيطرة عليها .

الكلمات المفتاحية: *Klebsiella pneumonia شديد الضراوة (hvkp) ، esherishia.coli (e.coli)، التهابات المسالك البولية (UTI) ، وسط زرعي tryptic soy broth (TSB)، محلول ملحي مخزّن من الفوسفات .(PBS)*

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Introduction

Klebsiella pneumoniae bacteria first isolated from lung of pneumonic died patients by Friedlander in (1882). Later, regarded as a saprophytic bacteria, not limited to lung, skin, but even can invade kidney and cause severe urinary tract infections [1].

The virulence factors have essential role in the pathogenicity of *K. pneumoniae* disorders like capsular polysaccharides type 1 & type 3 pili, factors involved in aggregative siderophores and adhesions [2]. Capsules have subdivisions can be catigorized into 77 serological kinds are necessary in *Klebsiella* virulence [3].

The capsular substances, forming fibrils modules that envelope the surface of bacteria, promote its from phagocytosis, on the other hand its prevent bacterial destruction by bactericidal agents in host serum via 2 types of pili [4]

Because of increase virulence ability, *K. pneumoniae* in the before antibiotic taking, considered important reason of community-acquired (CA) diseases in healthy hosts, involving chronic pneumonia typical types, particularly in patients with chronic diseases like diabetes mellitus. Later years community-acquired pneumonia via these pathogens are very rare, thus emerging of novel *K. pneumoniae* CA diseases, like liver abscess, several invasive infections caused by highly virulent klebsiella strains of particular kinds, as capsular type1 (K1) also urinary tract infections have been indicated [4،5].

The majority of adhesion and invasive ability of strains play gold role in the recurrence of diseases , *K. pneumoniae* have the ability to several antibiotic resistant. The invasive and adhesion ability that causing UTI is still controversial [5].

Really colonization of bacteria with its toxins and highly antibiotic resistance patterns considered main causing of healthcare-associated infections like urinary tract, wounds and burn infections. *Klebsiella* is the first one after *Escherichia coli* in nosocomial hospital-acquired bacteremia in addition to urinary tract infections (UTIs), affecting urinary catheterized patients. In fact, *K. pneumoniae* regarded as the main causative agent of infections indwelling-urinary catheters patients [6]. A high frequency of these bacterial UTI in patients was present in patients of high risks, like diabetic patients. As we know the several studies about bacteremia related intravascular catheters, on blood infections revealed that *Staphylococcus aureus* was the most one infections (30%), followed by *K. pneumoniae* (10%) [7]. In a recent years of meta-analysis studies on bacterial wound infections in hospitalized adult burn patients, *K. pneumoniae* reported that one of the most common Gram-negative rods, after *pseudomonas aeruginosa* [7] .

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Antibiotic Resistance

The emergence of chromosomal and extra chromosomal mutation genes that provide resistant ability to fluoro-quinolones makes that very difficult treatment of *K.pneumoniae* infections , so the last defense chance y only by carbapenems using [8].

Unfortunately multidrug-resistant *K. pneumoniae* (MDR) strains was evolved lastly and able to produce carbapenemase enzyme encoded by transmissible plasmid genes with high ability to distribution in medically important locations [8]. Several years ago carbapenemase particularly classes (A, B and D) which are β-lactamases have the ability to hydrolyzing all β-lactam antibiotic generations due to having encoded genes by transmissible elements like plasmids & transposons that rapidly diffused in bacteria, [9].

Biofilm and antibiotic resistance

K. pneumoniae could survive in the form of a thick biofilm layer in vitro, but clear in vivo biofilm studies were approved by Reid & coworkers in 1992. They used a scanning electron microscope to identify specific epithelial bladder cells in patients with spinal cord inflammation who had urinary tract infections caused by K. pneumoniae. [10]. *Klebsiella pneumoniae*, offer a good reason of nosocomial infections in many countries around the world, exclusively in immune-compromised patients and in indwelling medical devices involving grow of microorganisms as layers of biofilm. The secretion of broaded-spectrum beta-lactamases antibiotic resistance are enhanced by the fixed horizontal transfer of virulent genes that resistant to wide generations antibiotics via mobile elements such as transposons and plasmids which play effective role in *K. pneumoniae* nosocomial persistent infections [11].

According to bacterial research, K. pneumoniae and other bacteria grow inexorably on the surfaces of objects and tissues, generating thick biofilms that keep the germs there for a long time even in the presence of antibiotics and the immune system. Additionally, there is a possibility of bacterial biofilms spreading throughout the hospital and among patients. [12]. An essential factor of bacterial colonization in tissues & infection development is bacterial adhesion to host cells surface fimbrial and non-fimbrial factors. Fimbriae are external appendages comprised of many proteins of. *K. pneumoniae* that screte several virulence genes participate in pathogenecity, including lipopolysaccharide, capsular polysaccharide, fimbriae and serum resistance. *K. pneumoniae* strains has 3 different fimbrial kinds like mannose-resistant type 3 fimbriae, mannose-sensitive type 1

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fimbriae & the common pilus. All of these pili are participated in the adherence of *K. pneumoniae* species to host cells and in thick layers of biofilm production [13].

In this study, we can concentrate on the detection and investigation of virulence-associated traits such as antibiotic resistance, cell adherence, and biofilm production in a variety of highly pathogenic nosocomial K. pneumoniae strains isolates obtained from hospital admissions with urinary tract infections, some of which are in charge of recurrent outbreaks. [14].

Hypervirulent Klebsiella pneumonia

Hypervirulent *K. pneumoniae* (hvKp) is a recent *K. pneumoniae* variable strains that is clinically more virulent than original type of *K. pneumoniae* (cKp). *K. pneumoniae* is an opportunistic human pathogen responsible for nosocomial infections that is usually affect immune-compromised patients, whereas hypervirulent *K. pneumoniae* (hvKp) is clinically more aggressive wild type that reaches in diseases to healthy persons and can infect any site of the body. The genetic values lead to this patho - virulent type are involved in large plasmid and transmissible elements, Although (hvKp) is not susceptible to antibiotics effect, but its shown that can acquire evolutionary plasmids become multi-resistant and extensive resistant to a variety of antibiotics [15].

Materials and Methods

Laboratory Instruments and Equipment

Table (1): Laboratory Instruments and Equipment.

Instruments/Equipment	Company
Incubator	Fisher
Benson burner	Locally. Provided
Autoclave	Hirayama
Light microscope	LOMO
Sensitive electron balance	Precisa
Hood	Shinscang
Loop	Himedia

Chemical and Biological Materials

Table (2): Chemical and Biological Materials

Culture media	Company
Blood agar	Himedia
Mueller Hinton Agar	Himedia
TSI	HiMedia
IMVIC	HiMedia

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1- Sample collection

150 urine samples were collected from patients having urinary tract infections (UTI) admitted in several Baghdad hospitals like Ibn - cina, Al-kendy hospitals & specialized center of endocrinology in Baghdad city, then all specimens were cultured, diagnosed, and chemical tests were performed on them under sterile conditions to avoid contamination.

Inclusion criteria: All those patients attended to hospitals that suffering from severe and chronic urinary tract infections and urinary associated catheters, both males and female's adult.

Exclusion criteria: Urine samples from non-urinary tract infection patients, any bacterial isolates rather than *Klebsiella* should be excluded, children and those patients taking prescribed antibiotics.

2-Blood agar: Ordinary media for general purposes like colonies morphology and other growth features were prepared by standard procedures with 5% blood for preparation requiring.

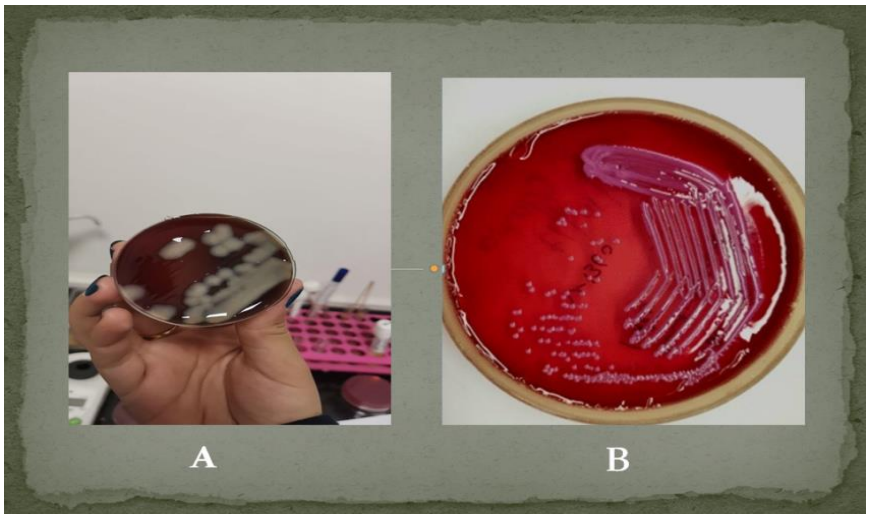

Fig (1): A- Klebsiella pneumonia, **B-** e. coli on blood agar.

3-Biochemical Test of *Klebsiellae pneumonia* and E. coli

Table (3): Biochemical tests of all isolates were given in this table.

Characteristics	Results of K. pneumonia	Figure	E. coli result	Figure
Indole	-ve	A	+ve	1
MR. (Methyl Red)	-ve	B	+ve	2
VP. (Voges Proskauer)	+ve	C	-ve	3
Citrate	+ve	D	-ve	4
TSIA (Triple Sugar Iron)	A\A	E	A\A	5

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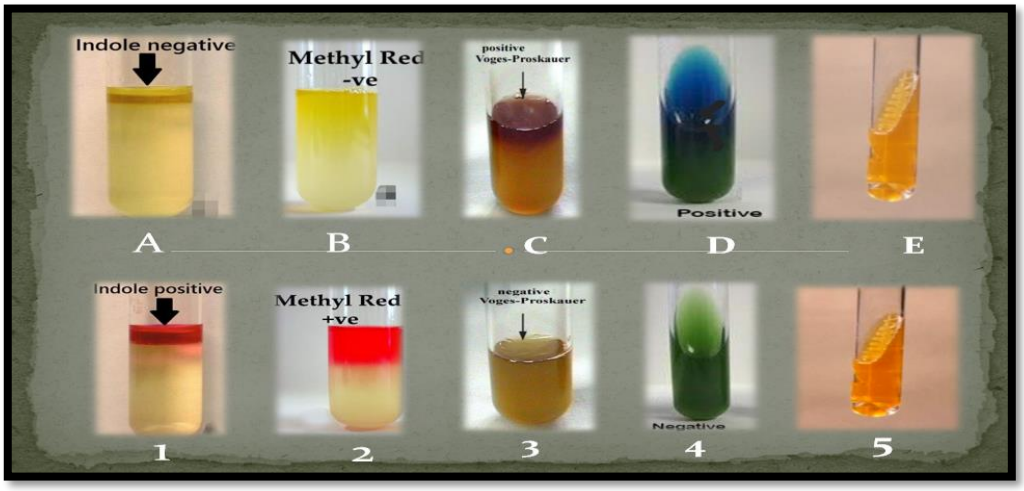

Fig (2): Biochemical Test (TSI & IMVIC).

4- Ensure bacteria diagnosis by VITEK 2 system

The VITEK 2 system provided recently in healthcare laboratories used for fast, accurate microbial identification, and antibiotic susceptibility testing. The innovative VITEK- 2 microbial investigation system includes effective database, that have automated stage available, rapid results, improved accuracy, with minimal time consuming. The VITEK- 2 system next generation stages provides large automated mode while high safety and eliminating manual operative procedures. The rapid time management give results provided more quickly than with manual microbial identification techniques. It is used for microbial identification, bacteria and yeast identification, antibiotic susceptibility testing (AST) and resistance mechanism detection [16،17].

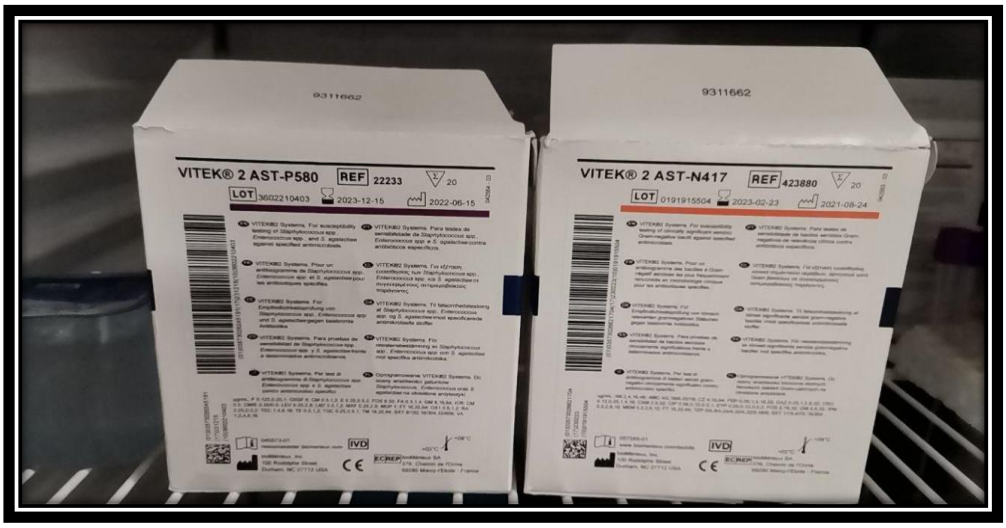

Fig. (3): vitek 2 system kit made in USA are used for identification of klebsella pneumonae spp.

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5- Biofilm assay

All Klebsiella pneumonia and E. coli isolates were tested for the presence of bacterial biofilm using the microtiter tissue culture plate method based on crystal violet assays. Bacteria were added to 10 ml of a special medium called tryptic soy broth (TSB), which was 0.2% glucose added for enhancement. The bacteria were then incubated overnight at 37 CO before being washed three times with sterile phosphate buffered saline (PBS) to remove non-adherent cells. The cells were then fixed in 200 l of methanol for 10 minutes. 250 ml of 100% ethanol was then added, and optical density was assessed at 570–600 nm using an ELISA reader after the plate had been stained with 200 ml of 1% crystal violet stain then left for 10 minutes. The cutoff point was 0.150 nm, so any results below the cutoff were negative, while any results equal to or greater than 0.90 nm were considered positive. One well was left with TSB medium only, which was considered as the negative control, and another well was left with broth and culture only, which was considered the positive control. [18،19].

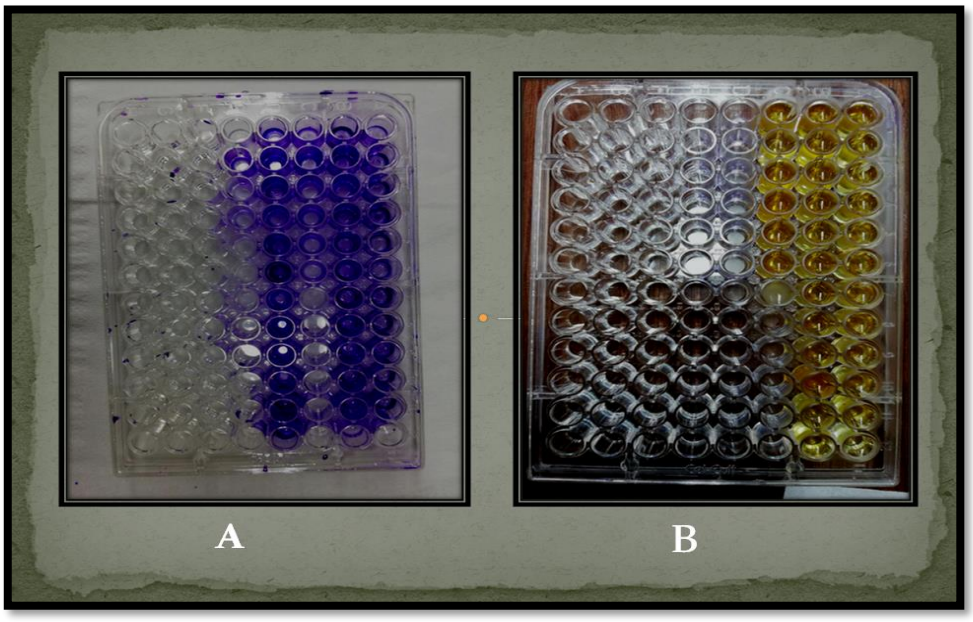

Fig. (4): The final step of biofilm formation in vitro.

6-Antibiotic Susceptibility Testing

The antibiotic susceptibility of *K. pneumoniae* isolates was determined by the Kirby–Bauer disk diffusion method. Suspension of each *K. pneumoniae* isolate was spread by sterile glass rods on the surface of *Mueller Hinton agar*. Then antibiotic discs were placed onto the surface of the inoculated *Mueller Hinton agar* plate, these antibiotics used were imipenem (10µg), ceftriaxone (20µg), gentamicin (10µg), amoxicillin (30µg) and amikacin (30µg). The plate was then incubated at

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35°C for 18 h. Antimicrobial susceptibility was determined by measuring the diameter of the inhibition zone according to clinical laboratory standard institutes (CLSI) [20].

Fig. (5): The antibiotic susceptibility on Mueller Hinton agar.

Statistical analysis

Data were analyzed statistically in cross- sectional study through (SPSS) version (25.0) in order to calculate association of bacterial frequency with percentage of antibiotic resistance and biofilm . *P* values < 0.05 were statistically significant.

Results

Table (1): samples isolated depending on gender.

Gender	K.pneumonea isolates	E.coli isolates	Total	p.value
Male	30	20	50	0.4 X^2=1.5
Female	70	30	100	
Total	100	50	150	

150 urine samples was isolated from patients with urinary tract infection, 100 samples UTI caused by ***Klebsiella pneumoniae***. 50 samples UTI caused by ***E. coli*** ,then 100 samples isolated from females (after diagnosis 70 samples diagnosed infection with *k. pneumoniae* and 30 samples diagnosis infection with E.coli) the others 50 samples isolated from males (after diagnosis 30 samples diagnosed infection with *k.pneumoniae* and 20 samples diagnosis infection with E.coli) at non-significant (p = 0.4) distribution.

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Table (2): diagnosis of bacterial samples.

Isolates	Blood agar	Vitec	Indole	MR	VP	CITRATE	TSI
K.pneumonea	100	100	-	-	+	+	A/A
E.coli	50	50	+	+	-	-	A/A
Total	150	150					

After samples isolated from patients were cultured on blood agar for basic culturing and diagnosed with IMVIC and TSI (biochemical tests)100 samples were diagnosed as *K. pneumonea* and 50 samples were diagnosed as *E. coli* the diagnosis was confirmed by vitek-2 system.

Table (3): bacterial biofilm formation.

K. pneumonia biofilm Cutoff (0.150)	(%) No.	E. coli biofilm Cutoff (0. 50)	(%) No.
normal ≤ 0.90	15 (15%)	normal ≤ 0.50	**35 (35%)**
Weak (0.90-0.150)	20 (20%)	Weak (0.50-0.100)	**30 (30%)**
Moderate (0.150-0.300)	24 (24%)	Moderate (0.100-0.200)	**20 (20%)**
High ≥ 0.300	41 (41%)	High ≥ 0.200	**15 (15%)**
Total	100 (100%)	Total	**100 (100%)**
p-value 0.00005		X^2=22.4	

By using an ELISA reader, the optical densities of *Klebsiella pneumonia* and *E. coli* were determined in the current study. *Klebsiella pneumonia's* biofilm cutoff point was 0.90 nanometers, while *E. coli's* was 0.50 nanometers, accounting for around 41% of the samples. In UTI patients, *Klebsiella pneumonia* isolates can produce strong biofilms, while 24% form moderate biofilms, 20% form weak biofilms, and 15% form normal biofilms. According to statistics, there are changes in Klebsiella pneumonia's ability to produce biofilms depending on the cutoff point (normal, weak, moderate, and strong) that are associated with UTI patients at (p=0.00005).

Table (4): Antibiotics resistance pattern.

Antibiotics	Dose micro gram	Sensitivity in NO(%)		Resistant in No (%)	
		k. pneumonia	*E.coli*	*k. pneumonia*	***E.coli***
Imipenem	10	59(59%)	48(96%)	41(41%)	**2(4%)**
Ceftriaxone	20	35(35%)	45(90%)	65(65%)	**5(10%)**
Gentamicin	10	35(35%)	47(94%)	65(65%)	**3(6%)**
Amoxicillin	30	15(15%)	35(70%)	85(85%)	**15(30%)**
Amikacin	30	15(15%)	25(50%)	85(85%)	**25(50%)**
Total %	-----	31.8 %	80%	68.2%	**20%**
p-value	0.00	X^2=33.4	(%of R)		

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In results of Antibiotic Susceptibility Testing imipenem inhabiting and killing 59% only of all but 41% of high biofilm producer bacteria resists imipenem. Ceftriaxone and gentamicin inhabiting and killing 35% of (normal and weak) biofilm producer bacteria. Amoxicillin and amikacin inhabiting and killing 15% of weak biofilm producer bacteria. High biofilm producer bacteria 41% were resist all Antibiotics with highly statistically significant differences of all antibiotic resistance percentage between *E. coli* & *K. pneumonia* at (p = 0.00).

Table (5): Antibiotic resistance of *K. pneumonea* isolates with biofilm ability.

Biofilm range	**Antibiotic resistance of *K.pneumonea* isolates with biofilm ability NO(%)**				
Antibiotic R%	IM	CEF	GE	AM	AK
normal ≤ 0.90	0	0	0	0	0
Weak (0.90-0.150)	0	0	0	20(20%)	20(20%)
Moderate (0.150-0.300)	0	24(24%)	24(24%)	24(24%)	24(24%)
High ≥ 0.300	41(41%)	41(41%)	41(41%)	41(41%)	41(41%)
Total	41(41%)	65(65%)	65(65%)	85(85%)	85(85%)

The ability of bacterial antibiotics resistant effected by biofilm producing (high biofilm producing enhance bacterial resistant of antibiotics) normal ≤ 0.90 were15% sensitive for all antibiotics, Weak (0.90-0.150) were 20% resist only low effecting antibiotics (Amoxicillin and Amikacin), Moderate (0.150-0.300) were 24% sensitive for only high effecting antibiotic (Imipenem IM). High ≥ 0.300 producing biofilm resist all antibiotics.

Discussion

Antimicrobial resistance (AMR) is a rapidly expanding problem in today's healthcare institutions all over the world. Through the synthesis of enzymes like Extended Spectrum -Lactamase (ESBLs) and Carbapenemase, *K. pneumoniae* has been found to develop antibiotic resistance more quickly than most bacteria. Additionally, K. pneumoniae is critical in the transfer of environmental bacteria's antibiotic resistance genes to clinically significant bacteria. Antimicrobial resistance has a variety of ways that will harm the effectiveness of treatment. Antibiotic resistance has been identified by the World Health Organization (WHO) as one of the top three global health issues. [21]. In this study 150 sample were isolate from patients with UTI according to the gender samples collected from females more than males, disagreed with study [Akter J, Chowdhury AMMA, Al FM. Study on prevalence and antibiotic resistance pattern of Klebsiella isolated from clinical samples in south east region of Bangladesh (American J Drug Discovery) [Dev. 2014;4:73–9.] [22], that reported males

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more infection with *k. pneumonae* than females also agreed with study [Isolation and Identification of *Escherichia coli* and *Klebsiella pneumonia*e by Tiemtoré RYW, Mètuor Dabiré A, Ouermi D, Sougué S, Benao S, SimporéJ., 20 June 2022] [23]. The same study reported *Klebsiella pneumoniae* caused UTI infections more than *E. coli* as our results shown 150 urine samples was isolated from patients with urinary tract infection 100 samples isolated from females (after diagnosis 70 samples diagnosed infection with *K. pneumoniae* and 30 samples diagnosis infection with E. coli) the others 50 samples isolated from males (after diagnosis 30 samples diagnosed infection with *K.pneumoniae* and 20 samples diagnosis infection with *E.coli*). [24]. In our study, 65% of K. pneumoniae develop moderately high levels of biofilm, compared to 35% who produce biofilm at normal or weak levels. According to Hassan et al., out of 110 isolates of K. pneumoniae examined, 70 (64.7%) were found to be strong or moderate biofilm producers, while 40 (35.3%) were found to be weak producers.

According to Seifi et al. [25], the majority of K. pneumoniae (93.6%) produced biofilms, while just 6.4% were not biofilm producers (Normal). Hypervirulence and carbapenem resistance have emerged as two distinct evolutionary directions leading high-risk *Klebsiella pneumoniae* lineages to epidemic success so in our study shown 41% of *K. pneumoniae* (high biofilm producer) were resist impenem. Imipenem inhabiting and killing all (normal, weak and moderate) biofilm producer bacteria 59%. Ceftriaxone and gentamicin inhabiting and killing (normal and weak) biofilm producer bacteria about 35%. Amoxicillin and amikacin inhabiting and killing weak biofilm producer bacteria 15%. Journal of Global Antimicrobial Resistance shown carbopenem resistant klebsiella have been increasingly reported worldwide. According to the China Antimicrobial Surveillance Network, the meropenem resistance rate in *K. pneumoniae* increased from 2.9% in 2005 to 26.8% in 2019. According to the European Centre for Disease Prevention and Control (ECDC) [global perspective on the convergence of hypervirulence and carbapenem resistance in *Klebsiella pneumoniae* by Peng Lan, Yan Jiang, Jiancang Zhou, Yunsong Yu., Journal of Global Antimicrobial Resistance 25 (2021) [26].

Conclusion

In this study we can conclude that in the present years establishing a very drug resistant strains of *Klebsiella pneumoniae* called hypervirulent strains due to the emergence and acquisition of new resistance plasmid and chromosomal genes that resistant to highly effective antibiotics like imipenem and carbopenem as well as become multiple and extensive drug resistance particularly in elderly

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suffering from chronic infections like long catheter related UTI and other diseases that contribute to a big nosocomial hospitalized troubleshooting cases.

Recommendations

We recommend in our study further future studies in a big researches concerning the molecular genetic studies targeting the virulent genes of these hypervirulent bacterial strains by PCR techniques as a good strategies for treatment as well as molecular sequencing studies for imipenem resistance strains.

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