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Detection of blaOXA-48 Gene in Carbapenem-Resistant Klebsiella Pneumoniae and Escherichia Coli from Urine Samples

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Abstract

Gram-negative bacteria have been established in Egypt to have high rates of antimicrobial resistance. A major threat to human health exists in every country due to the emergence and spread of carbapenem-resistant Gram-negative bacteria. Hundred and twenty-seven Gram-negative bacterial isolates were identified and their antibiotic susceptibility testing were performed. They include 100 *K. pneumoniae* and 27 *E. coli* isolates. Phenotypic identification of carbapenemase production was confirmed by Modified Hodge test (MHT) and Modified Carbapenem Inactivation Method (mCIM). The *bla*OXA-48 gene was detected using two variant primers (OXA-438 and OXA-743) conventional polymerase chain reaction. The antimicrobial susceptibility test for *K. pneumonia*e and *E. coli* showed 100% resistance to ampicillin, amoxicillin, pipracillin/tazobactam, cefotaxime, cefepime, ceftazidime, imipenem, meropenem, ciprofloxacin, ofloxacin and nitrofurantoin. *K. pneumonia*e also possessed resistances reached 91% to gentamycin, 86% to co-trimoxazole and 79% to amikacin. On the other hand, 100% of *E. coli* isolates were resistant to co-trimoxazole. Additionally, they exhibited a resistance to gentamycin (81.5%) and amikacin (77.8%). Genotypic study using multiplex PCR for detection of the *bla*OXA-48 gene (by two variant primers: OXA-438 and OXA-743) revealed that among the 100 *K. pneumoniae* isolates, 22% and 18% were detected using OXA-438 and OXA-743, respectively. Similarly, the 27 carbapenem resistant *E. coli* isolates revealed the presence of 7.4% of both OXA-438 and OXA-743 primers.

Keywords: Carbapenem, Escherichia coli, Klebsiella pneumoniae, blaOXA-48.

Introduction

The drug resistance is the ability of microorganisms to withstand the effects of antimicrobial drugs, and it happens when an antibiotic loses its ability to effectively stop the microbial growth (Beceiro et al., 2013; Nadeem et al., 2020). Antibiotic resistance is a growing global challenge to both human health and the medical treatment of disease (Allen et al., 2010). Global healthcare systems are under a great deal of pressure due to antibiotic resistance.

The carbapenems family is one of the most important classes of antibiotics which used to treat bacterial infections brought on by multiple drug resistant (MDR) of Gram-negative Enterobacteriacae (Codjoe & Donkor, 2017). Extensive and irrational usage of carbapenems in hospitals and other medical settings have prompted the establishment and spread of bacteria that are resistant to carbapenem. They can only be treated with antimicrobial medications like tigecycline and colistin (Codjoe & Donkor, 2017; Neuner et al., 2011), that are regarded as last-resort treatments for treating Enterobacteria that are resistant to carbapenem (CRE) (Huttner et al., 2012).

The mechanisms govern Gram-negative bacteria's resistance to carbapenems, including the production of carbapenemases and the efflux pumps of carbapenems (Codjoe & Donkor, 2017; El-Badawy et al., 2019). For the definition purposes provided by the Centers for Disease Control and Prevention, when *K. pneumoniae* displays resistance to at least one drug from three or more classes of antimicrobial agents, it is referred to as MDR (Wolfensberger et al., 2019). MDR bacteria that become resistant to all antibiotic classes other than two or fewer classes are said to have extensive drug resistance (XDR) (Pattnaik et al., 2019). In contrast to XDR bacterial isolates, pan drug resistance (PDR) refers to bacteria that become resistant to all antibiotics including tigecycline and polymyxin (El-Badawy et al., 2019; Pattnaik et al., 2019).

Carbapenems Resistance Enterobacterales (CRE) has become a major global concern due to the production of carbapenemase, a class of enzyme that hydrolyzes all β-lactam antibiotics, including carbapenem. (Filgona et al., 2018; Jean et al., 2018; Mariappan et al., 2017). Carbapenemases of CRE can be differentiated into three classes A, B, and D according to Ambler (1980). Furthermore, Bush & Jacoby (2010) stated that β-lactamases which are inhibited by boronic acid or clavulanic are a member of carbapenemases that belong to class A. Metallo-lactamases are belonging to class B and include those that are inhibited by dipicolinic acid and EDTA; and are able to hydrolyze all β-lactams except aztreonam. Class D includes all OXA-48-like β-lactamases (oxacillinases) such as OXA-48 , OXA-72, and OXA-244, which are able to weakly or not at all hydrolyze cephalosporins but can hydrolyze carbapenems, and which are not inhibited by conventional inhibitors (Nordmann et al., 2011; Nordmann et al., 2012; Mairi et al., 2018).

The most important enzymes of Class A is *K. pneumoniae* carbapenemase (KPC), and Class B are New Delhi metallo β-lactamase (NDM), Verona integron-encoded metallo- β-lactamase (VIM), Imipenemase (IMP). The Class D included oxacillinase OXA-48 and its variants. However, among Enterobacteriaceae, OXA-48 and NDM-1 are the most frequently reported carbapenemases, particularly in *E. coli* and *K. pneumoniae* (Filgona et al., 2018) .

Carbapenemase genes often encoded in plasmids or mobile DNA elements that facilitate their transfer and spread among bacterial strains (Mathers et al., 2011). OXA-48, NDM, KPC, IPM and VIM are some examples of the most common antibiotic resistant genes which widely distributed among Enterobacteriaceae strains. The *bla*OXA-48 gene is first discovered in carbapenem-resistant *K. pneumoniae* isolates in Istanbul, Turkey. It has now received widespread media coverage for its role in nosocomial infection epidemics across the world, especially from the Mediterranean region's surrounding countries (Carrër et al., 2008; Mathlouthi et al., 2017; Mairi et al., 2018). North African countries (like Egypt, Morocco, Tunisia and Libya) are thought to be the major reservoirs for *bla*OXA-48 gene producing Enterobacteriaceae (Benouda et al., 2010; Poirel et al., 2012; Mathlouthi et al., 2017; Stewart et al., 2018).
In this study, the distribution of carbapenem-resistant *K. pneumonia* and *E. coli* would be monitored using multiplex PCR for OXA-438 and OXA-743 primers in order to manage their spread.

Material and Methods

Sample Collection and Identification of Isolates

This cross-sectional research was carried out between January to December 2021 at Urology and Nephrology Center, Mansoura University. The study population consisted of hospitalized patients from all departments who were admitted to the hospital with suspicions of urinary tract infection. The urine samples culture was cultured on CLED agar (cystine–lactose–electrolyte-deficient agar) and 5%

Columbia blood agar plates (Oxoid). The plates underwent an overnight aerobic incubation at 37°C following inoculation. Identification of isolates and antibiotic susceptibility testing (AST) were performed by using Vitek2 compact system (BioMerieux, France). The selected antibiotics were ampicillin 500mg, amoxicillin/clavulanic acid 250/125mg, cefepime 2g/100ml, cefotaxime 1gm, ceftazidime 1gm, meropenem 1gm, imipenem 500mg, gentamicin 10mg/1ml, ciprofloxacin 500mg, ofloxacin 200mg, trimetropim/ sulfametoxazol 160/800mg, amikacin 500mg/2ml, piperacillin/tazobactam 4.5gm and nitrofurantoin 100mg. Clinical Laboratory Standards were used to interpret the minimum inhibitory concentration (MIC) data.

Screening for Carbapenemase Production

Carbapenem non-susceptible (including resistant and intermediate) isolates were confirmed using MICs; IPM ≥ 2(μg ⁄ml) and MEM ≥ 2 (μg ⁄ml) according to CLSI, Clinical and Laboratory Standard Institute (2022).

Modified Hodge Test (MHT)

The MHT involved the subsequent steps: suspension preparation of an overnight culture of carbapenem susceptible *E. coli* reference strain (*E. coli* ATCC 25922) in 3 ml saline solution adjusted to 0.5 McFarland standards. The *E. coli* was inoculated homogenously using a sterile cotton swab on a Muller Hinton agar (MHA) plate surface. After drying, a 10μg meropenem antibiotic disc was placed on the middle of the plate. The tested isolate was streaked and incubated overnight at 37°C. Quality control of the meropenem disc was performed according to CLSI protocol with each run using: MHT-Positive *K. pneumonia* (ATCC BAA-1705) and MHT-Negative *K. pneumonia* (ATCC BAA-1706). After incubation, the inoculated plates were examined for a clover leaf-like pattern at the intersection of the tested isolate, and therefore the *E. coli* (ATCC 25922) within the inhibition zone of the meropenem disc. Enhanced growth of the meropenem susceptible *E. coli* and *K. pneumoniae* is an indicator for carbapenemase production (Centers for Disease Control and Prevention, 2019).

Modified Carbapenem Inactivation Method (mCIM)

The mCIM test was performed according to CLSI (2021). A 1-μL of fresh bacterial colonies was suspended in 2ml of tryptic soy broth (Merck, Darmstadt), then a 10μg meropenem disc (Bioanalyse) was applied and the plates were incubated for four hours at 35 °C ±2 °C. After 18 to 24 hours, the meropenem disc was placed on Mueller-Hinton agar medium spread with 0.5 McFarland of *E. coli* ATCC 25922. Isolates exhibited inhibition zone diameter of 6mm to 15mm, were considered to have carbapenemase activity, while strains with a zone diameter of 19mm were considered to have negative activity, according to CLSI criteria.

Combined disc test (CDT)

0.5 McFarland of an overnight culture of the tested isolate was performed on Muller Hinton agar, according to Yong et al. (2002). On inoculated plates, two 10 μg imipenem antibiotic discs were used (one imipenem disc alone and the other with 10 μl of a 0.5 M EDTA solution). After incubation at 37°C for 16 to 18 hours, the test was considered as positive if the zone of inhibition of imipenem + EDTA discs was >7 mm compared to imipenem disc alone.

DNA Extraction and Amplification

Total DNA was extracted from bacterial cells using High Pure PCR Template Purification Kit, (Germany). PCR amplifications were carried out on a Thermal Cycler 9700 instrument (PERKINELMER) using the Gene Amp kit. For gene amplification, 3μL extracted DNA, 12.5μL master mix, 7.5μL nuclease-free water and 1μL of each forward and reverse primers were added to a final mixture volume of 25μL. The two primer pairs used to detect the OXA-48 gene were OXA-438F (5′-GCGTGGTTAAGGATGAACAC -3′) and OXA-438R (3′-CATCAAGTTCAACCCAACCG-5′) resulting size of PCR product is 438 bp (Poirel et al., 2011); and OXA-743F (5′ TTGGTGGCATCGATTATCGG -3′) and OXA-743R (3′-GAGCACTTCTTTTGTGATGGC -5′) with expected size of PCR product 743 bp (Mihajlović-Ukropina et al., 2016). The thermal

cycling protocol started with an initial denaturation step at 94°C for 10 minutes, then 40 cycles included denaturation at 94°C for 40 seconds, annealing at 54°C for 40 seconds and extension at 72°C for 1 minute. A final extension step was at 72°C for 7 minutes. The amplified products were ran on to 2% agarose gel electrophoresis and ethidium bromide (0.5 µg/mL) at 70V. The gel was exposed to a UV light for photo documentation. The amplified product's molecular weight was estimated using a 1000 bp DNA marker (Thermo Scientific).

Statistical Data Analysis

Excel spreadsheet and SPSS software (version 24) were used for statistical analysis. Statistical analysis was conducted using chi-square tests and p-value <0.05 were considered statistically significant.

Results

Antibiotic Susceptibility

Out of 127 CRE isolates, 100 (78.7%) were *K. pneumoniae*, while 27 (21.3%) isolates were other types including *E. coli.* AST showed that 100% (100/100) of isolates exhibited XDR pattern, while none of the isolates exhibited PDR pattern. The carbapenem resistant *K. pneumoniae* CRKP clinical isolates were 100% resistant to amoxicillin, ampicillin, piperacillin/tazobactam, cefotaxime, cefepime, ceftazidime, imipenem, meropenem, ciprofloxacin, ofloxacin and nitrofurantoin. Gentamycin resistance was 91% and Co-trimoxazole 86%, while amikacin exhibited 79% of resistance as shown in Table1 and Figure 1.

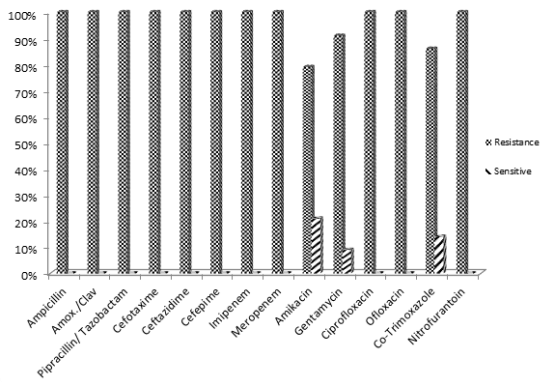

Figure 1. Antibiotic susceptibility test for the *K. pneumoniae* isolates.

Table 1. The antibiotic susceptibility test for the *K. pneumoniae* and *E. coli* isolates**.**

Antibiotic	*K. pneumoniae*		*E. coli*	
	Resistance	Sensitive	Resistance	Sensitive
Ampicillin 500mg	100%	0%	100%	0%
Amox./ clavulanic 250/125mg	100%	0%	100%	0%
Pipracillin/ Tazobactam 4.5gm	100%	0%	100%	0%
Cefotaxime 1gm	100%	0%	100%	0%
Ceftazidime 1gm	100%	0%	100%	0%
Cefepime 2g/100ml	100%	0%	100%	0%
Imipenem 500mg	100%	0%	100%	0%
Meropenem 1gm	100%	0%	100%	0%
Amikacin 500mg/2ml	79%	21%	77.8%	22.2%
Gentamycin 10mg/1ml	91%	9%	81.5%	18.5%
Ciprofloxacin 500mg	100%	0%	100%	0%
Ofloxacin 200mg	100%	0%	100%	0%
Co-Trimoxazole 160mg/800mg	86%	14%	100%	0%
Nitrofurantoin 100mg	100%	0%	100%	0%

The carbapenem resistant *E. coli* showed that 100% were resistant to ampicillin, amoxicillin, piperacillin/tazobactam, cefotaxime, cefepime, ceftazidime, imipenem, meropenem, ciprofloxacin, ofloxacin, nitrofurantoin and Co-trimoxazole. Resistance of gentamycin and amikacin was 81.5% and 77.8%, respectively as represented in Table1 and Figure 2.

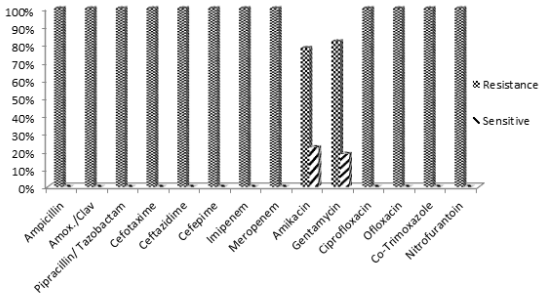

Figure 2. Antibiotic susceptibility test for the *E. coli* isolates.

MHT and mCIM test

The result of Modified Hodge Test

(MHT) showed that 72% of *K. pneumoniae* and 81.5% of *E. coli* were positive for MHT. On the other hand, There are 44% of *K. pneumonia* and 37% of *E. coli* were positive for mCIM test as indicated in Table 2.

Multiplex PCR of blaOXA-48

Figure 3 showed representative positive samples for amplification of *bla*OXA-48 gene using a multiplex PCR for both OXA-438 and OXA-743 primers. Lanes 1 and 8 exhibited single 743 bp DNA fragment. Lanes 2, 3and 6 possessed a single 438 bp DNA fragment. Lane 4, 5, 7 and 9 exhibited the amplification of both DNA fragments corresponding to OXA-438 and OXA-743 primers.

The Data analysis of the resulting PCR products revealed that OXA-438 primers annealed with 22% and 7.4% of *K. pneumonia* and *E. coli* isolates, respectively. Furthermore, the OXA-743 annealed with 18% and 7.4% of *K. pneumonia* and *E. coli* isolates, respectively (Table 2).

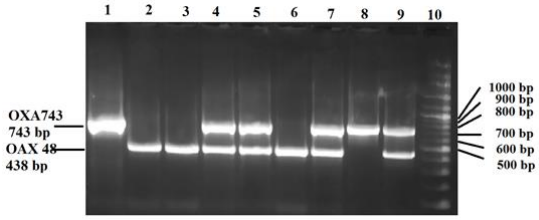

Figure 3. Agarose gel electrophoresis profile of the multiplex PCR products of clinical *K. pneumoniae* and *E. coli* isolates. Lane 1-5, multiplex PCR patterns for *K. pneumoniae*. Lane 6-9, multiplex PCR patterns for *E. coli*. Lane 10, 1000 bp marker.

Table 2. The general results of phenotypic (MHT & mCIM test) and genotypic (OXA-48 gene) characters of *K. pneumoniae* and *E. coli* isolates.

Isolates	Phenotype		Genotype	
	MHT	mCIM	OXA-438	OXA-743
K. pneumoniae	72 (72%)	44(44%)	22(22%)	18(18%)
E. coli	22(81.5%)	10(37%)	2(7.4%)	2(7.4%)

Phenotypic and Genotypic Characteristic Patterns of Bacterial Isolates

The phenotypic characteristics (based on MHT and mCIM tests) associated with the genotypic characteristics (based on PCR using OXA-438 and OXA-743 primes) for both of *K. pneumoniae* and *E. coli* isolates, were represented in Tables 3 and 4, respectively. The results exhibited many different patterns for distribution of those four characters (MHT, mCIM, OXA-438 and OXA-743) among the isolated bacteria as described within Tables 3 and 4.

Table 3. Phenotypic and genotypic characteristic patterns of the carbapenem-resistance *K. pneumoniae* isolates.

Number of cases	Phenotype		Genotype	
	MHT	mCIM	OXA-438	OXA-743
5	-	-	+	+
4	+	-	+	+
3	+	-	+	-
6	+	+	+	+
28	+	+	-	-
28	+	-	-	-
1	-	+	+	+
1	-	+	-	+
1	+	+	-	+
4	-	+	-	-
2	+	+	+	-
1	-	+	+	-
16	-	-	-	-

Table 4. Phenotypic and genotypic characteristic patterns of the carbapenem-resistance *E. coli* isolates.

Number of cases	Phenotype		Genotype	
	MHT	mCIM	OXA-438	OXA-743
2	+	-	+	+
12	+	-	-	-
8	+	+	-	-
2	-	+	-	-
3	-	-	-	-

Discussion

To address the antimicrobial resistance (AMR) crisis, an international action plan with five primary goals was agreed by the WHO conference (WHO, 2015). Through observation and study, one objective is to increase the body of knowledge and evidence. The causes of antimicrobial resistance (AMR) may be tracked using surveillance data, which can also show patterns in all the other sectors (Gandra et al., 2020; Peng et al., 2019). The analysis of such data thus supports the implementation of infection control programs (El-Kholy et al., 2020; Jayatilleke, 2020). Along with influencing the selection of resistance testing procedures and treatment choices (Nordmann & Poirel, 2019). Egypt's Healthcare-associated Infections (HAI) surveillance system, established in 2011, is the Eastern Mediterranean Region (EMR) of WHO's

leading prospective, standardized system (See et al., 2013; Talaat et al., 2016). However, just a few studies using the surveillance data from the system have documented the rising prevalence of CRE in Egypt (Kotb et al., 2020). The academic network, on the other hand, tried to contribute research data to close the knowledge gap regarding the severity of the CRE problem in Egypt (El-Kholy et al., 2021). In many clinical laboratories, routine testing for CRE resistance mechanisms is currently not essential (Singh-Moodley & Perovic, 2016). As a result, they are unable to distinguish between resistance brought on by various mechanisms, such as the over expression of AmpC enzymes and membrane impermeability, and carbapenem non-susceptibility brought on by the development of carbapenemases. One of the most troublesome bacterial infections is *K. pneumoniae*, which frequently displays the MDR pattern. Hospitalized patients with *K. pneumoniae* had higher mortality and morbidity rates, particularly in acute care facilities like cardiac care units and intensive care (Moemen & Masallat, 2017). In our study, there are higher frequencies of XDR *K. pneumoniae* infection100 (78.7%) were *K.* pneumoniae, while 27 (21.3%) isolates were other types including XDR *E. coli*. The bacterial pathogen that was most frequently found was *K. pneumoniae* (78.7%), followed By *E. coli* (21.3%). Haji et al. (2021) found that the most common bacterial pathogen found was *E. coli* (44%), followed by *K. pneumoniae* (20%).

However, greater rates of *K. pneumoniae* infection have already been noted in several investigations in Egypt (Ghaith et al., 2020; Mohsen et al., 2017). Frequently, the prolonged and excessive use of several antibiotics results in the development of XDR strains (Maseda et al., 2014). Public health is gravely threatened by the global dissemination and increased incidence of these XDR strains in clinical settings (Bi et al., 2017). The alarming prevalence of XDR *K. pneumoniae*, XDR *E. coli* which were 100%, and resistance to all but one or two of the tested antibiotics were described here. According to earlier reports, ampicillin and cephalosporin resistance rates in Egypt were high, while carbapenem and quinolone susceptibility rates were higher (Fahmey, 2013; Moore et al., 2005; Shehab El-Din et al., 2015). The fact that ampicillin and cephalosporin are used as the first and second lines of empirical treatment in Egypt is the cause of the significant resistance to these antibiotics. In our investigation, more than 85% of the isolated *K. pneumoniae* and 79.65% for *E. coli* exhibited resistance to aminoglycoside, which is a very high level of resistance. Additionally, this study found a very high rate of carbapenem resistance, with a rate of 100%. Our findings revealed a high prevalence of extensive drug resistance (XDR) *E. coli* (100%) which was higher than results obtained by Abdelaziz et al. (2021). Also, a high prevalence of MDR *E. coli* (73%) was recoded (Masoud et al., 2021).

Increased carbapenem resistance was observed in the current investigation was observed in *K. pneumoniae* (100%) and in *E. coli* isolates (100%). Our results are higher than those of El-Shaer et al. (2021) who observed that carbapenem resistance was 12.5% for meropenem and 6.9% for imipenem. Also, it was higher than A different Egyptian investigation at Alexandria Main University Hospital reported that 240 out of 706 (33.9%) Enterobacteriaceae isolates were ertapenem resistant (Iman et al., 2016). In another study, it was found that 45% of the Enterobacteriaceae isolates that obtained from patients hospitalized to the Prince Sultan Military Medical City (Riyadh, Saudi Arabia) were resistant to ertapenem (AlTamimi et al., 2017).

In this study, out of 100 and 27 phenotypic carbapenem bacterial isolates of *K. pneumoniae* and *E. coli*, respectively, were positive on the MHT by 72%) and 81.5%, respectively. ElMahallawy et al. (2018) found that when employing MHT to determine the activity of carbapenemases, 49% of the isolates were carbapenemase producers. The modified Carbapenem Inactivation Method (mCIM) was used to test *K. pneumoniae* isolates and *E. coli* isolates that did not exhibit sensitivity to at least one carbapenem for the presence of carbapenemase. This technique has more than 99% sensitivity and specificity for carbapenemase detection among Enterobacteriaceae isolates, according to CLSI (2018) recommendations. In this study, the modified Carbapenem Inactivation Method (mCIM) test exhibited carbapenemase positive results reached 44% and 37% for *K. pneumoniae* and *E. coli* isolates, respectively. The investigation performed by Li et al. (2017) revealed that 100% carbapenem- resistant *K. pneumoniae* isolates were positive for mCIM test. On the other hand, 71.8% were

carbapenemase positive using mCIM test for *K. pneumoniae* isolates (Awoke et al., 2022).

In the Mediterranean region of Africa and Europe, *bla*OXA-48 gene is frequently found in most of Enterobacteriaceae (Sekyere et al., 2016). This study revealed that 22% and 18% of carbapenem-resistant *K. pneumoniae* isolates gave positive amplification when the OXA-438 and OXA-743 primers were used, respectively. Additionally, carbapenem-resistant *E. coli* isolates exhibited 7.4% positive PCR products when both previous primers were used. El-Domany et al. (2021) found that *bla*OXA-48 was detected in 52.0% of *K. pneumoniae* isolates.

In clinical Gram-negative bacteria from Egyptian tertiary care facilities, our study found worrisome rates of resistance. These high rates of resistance underline the necessity of ongoing resistance monitoring, adherence to infection control guidelines, and the urgent need for Egypt to create a national antimicrobial stewardship plan.

Conclusion

This study revealed presence of strong (extensive drug resistance) XDR within CR-*K. pneumoniae* and CR-*E. coli* isolates. Furthermore, the associated multiplex PCR detection of *bla*OXA-48 gene within those clinical isolates supports the necessity for ongoing and regular screening for CR *K. pneumoniae* and *E. coli* isolates in order to prevent the spread of those XDR strains.

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الملخص العربي

عنوان البحث: الكشف عن جين blaOXA-48 في Klebsiella pneumoniae و Escherichia coli المقاومة للكاربابينيم من عينات البول

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تم إثبات أن البكتيريا سالبة الجرام تتمتع بمعدلات عالية من المقاومة للمضادات الميكروبية في مصر، حيث يوجد تهديد كبير لصحة الإنسان في كل البلاد بسبب ظهور وانتشار البكتيريا سالبة الجرام المقاومة للكاربابينيم، فى تلك الدراسة تم تشخيص ١٢٧ عزلة بكتيرية سالبة الجرام و تم إجراء لها اختبار الحساسية للمضادات الحيوية، قد اظهرت النتائج أن ١٠٠ عزلة كانت من بكتيريا K. pneumoniae الكليبسيلا الرئوية ، و٢٧ عزلة كانت من E. coli الإشريكية القولونية، كما تم تأكيد التحديد المظهري لإنتاج الكاربابينيماز عن طريق اختبار هودج المعدل (MHT) وطريقة تعطيل الكاربابينيم المعدلة (mCIM)، أيضا تم الكشف عن جين blaOXA-48 باستخدام بادئين مختلفين (OXA-438 وOXA-743) بواسطة تقنية تفاعل البلمرة المتسلسل، أظهر اختبار الحساسية المضادة للميكروبات أن الكليبسيلا الرئوية والإشريكية القولونية مقاومة بنسبة ١٠٠٪ للأمبيسلين، أموكسيسيلين، بيبراسيلين/تازوباكتام، سيفوتاكسيم، سيفيبيم، سيفتازيديم، إيميبينيم، الميروبينيم، سيبروفلوكساسين، أوفلوكساسين ونيتروفورانتوين، كما امتلكت الكليبسيلا الرئوية أيضًا مقاومة بلغت ٩١٪ للجنتاميسين، و٨٦٪ للكوتريموكسازول و٧٩٪ للأميكاسين، ومن ناحية أخرى، كانت ١٠٠٪ من عزلات الإشريكية القولونية مقاومة للكوتريموكسازول، بالإضافة إلى ذلك، أظهروا مقاومة للجنتاميسين (٨١,٥٪) والأميكاسين (٧٧,٨٪)، و قد كشفت دراسة النمط الجيني باستخدام تفاعل البلمرة المتسلسل المتعدد للكشف عن جين blaOXA-48 (بواسطة بادئين مختلفين: OXA-438 وOXA-743)، أنه من بين ١٠٠ عزلة من الكليبسيلا الرئوية ، تم اكتشاف ٢٢٪ و١٨٪ باستخدام OXA-438 وOXA-743 على التوالي، وبالمثل، كشفت عزلات الإشريكية القولونية لـ ٢٧ عزلة مقاومة للكاربابينيم عن وجود ٧,٤٪ باستخدام البادئات OXA-438 وOXA-743.