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Aminoglycosides Resistance Gene Detection in *Klebsiella pneumoniae* and *Escherichia coli* by Multiplex PCR

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ABSTRACT

Aminoglycosides (AMG) are a significant class of antibiotics frequently used with ß-lactams in the management of severe infections brought on by both Gram-negative and Gram-positive bacteria. The clinical efficacy of these antibiotics is currently under jeopardy due to rising Gram-negative bacteria's aminoglycoside resistance. An significant insight into the possible difficulties of treating bacteria comes from the characterization of the gene profiles for antibiotic resistance. *E. coli* and *K. pneumoniae* were subjected to a rapid-multiplex-PCR assay to look into the genes producing aminoglycoside-modifying enzymes (AMEs) and assess how common these resistance genes were. The disc diffusion method (Kirby-bauer) was used to assess the antimicrobial susceptibility of 95 bacterial strains against gentamicin, amikacin, neomycin, and tobramycin, with 54 strains of *K. pneumoniae* and 41 strains of *E. coli* making up the total. Three distinct sets of primers, *aph*(3), *ant*(2), and *aac*(6), were used in standard multiplex PCR to target AMG resistance genes. Ten distinct resistance patterns for *E. coli* and *K. pneumoniae* were found after an analysis of antimicrobial susceptibility tests against AMG for tested bacteria. The most common AME-genes in *K. pneumoniae*, according to the mPCR, were *aac*(6) and *ant*(2) (77.78% for both), followed by *aph*(3) (48.15%). While among *E. coli* isolates, *aac*(6) (75.61%), *ant*(2) (56.1%), and *aph*(3) (48.78%) had the highest prevalence of AME-gene resistance. Each isolate of *E. coli* and *K. pneumoniae* gained one or more drug-resistant genes, according to the results of the multiplex-PCR. It was hypothesised that the same resistance gene was horizontally spread between other bacterial species.

Keywords: Aminoglycosides resistance; *aph*(3) gene; *ant*(2) gene; *aac*(6) gene; *Escherichia coli*, *Klebsiella pneumoniae.*

INTRODUCTION

Aminoglycosides (AMG) are a type of antibiotics that are frequently used with ß-lactams to treat severe infections brought on by Gram-negative and Gram-positive bacteria. The clinical effectiveness of these antibiotics is currently in danger due to rising AMG resistance among Gram-negative bacteria. In Gram-negative bacteria, the emergence of aminoglycoside-modifying enzymes (AMEs) and 16S rRNA methylases results in the development of aminoglycoside resistance (Ramirez and Tolmasky, 2010; Wachino and Arakawa, 2012). Aminoglycosides are potent antibiotics with bactericidal effects that attach to the bacterial cell's ribosome and inhibit the production of proteins. Resistance to such antibiotic groups is primarily mediated by the development of enzyme modifications such as acetyltransferase, phosphortransferases, and adenyltransferases, modification of the target site as a result of a mutation in the 16s rRNA or ribosomal proteins (Yamane *et al*., 2005; O'Connor *et al.*, 1991), and decreased intracellular antibiotic accumulation resulting from a change in outer membrane (Magnet *et a*l., 2001).

There are three types of AMEs (Shaw *et al*., 1993; Davies & Wright, 1997; Wright & Thompson, 1999), N-Acetyltransferases (AAC, catalyzes acetyl-CoA-dependent acetylation of amino group), O**-**phosphotransferases (APH catalyzes ATP-dependent phosphorylation fahydroxyl group), and O-Adenyl-transferases (ANT, catalyzes ATP-dependent adenylationofahydroxyl group). Characterization the profiles of antimicrobial resistance gene distribution provide important information on the potential difficulty of treatment of bacteria. This information can be employed for facilitating prompt and effective treatment of bacterial infection. To examine the prevalence of AMG resistance gene, several methods have been developed, including conventional single-PCR and multiplex-PCR assays combined with agarose gel electrophoresis analysis, hybridization with DNA probes, and sequence analysis (Clark *et al*., 1999; Vakulenko *et al*., 2003, Kishk *et al*., 2021). The existing methods have some disadvantages, such as how time-consuming, labor-intensive, and difficult it is to simultaneously evaluate many genes.

A flexible framework for evaluating thousands of possible antimicrobial resistance genes simultaneously is provided by DNA chips (Disney *et al*., 2004; Chen *et al*., 2005). On the other side, discovering many clinical isolates during an epidemiological research is expensive and time-consuming. Therefore, a rapid, low-cost, high-throughput approach is required to analyse the distribution of AMG resistance genes in clinical isolates. The polymerase chain reaction (PCR) method is faster, more sensitive, and more focused for this type of detection when compared to southern blot hybridization, macrorestriction, fingerprinting, and MIC determination (Vanhoof *et al*., 1994). Meanwhile, numerous genes can be identified concurrently by multiplex-PCR, and m-PCR tubes have the benefit of quickly and accurately detecting genotypic resistance to a variety of drugs (Geha *et al*., 1994; Martineau *et al*., 2000). For that reason, the goal of this research

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was to provide a quick multiplex-PCR (mPCR) test for the simultaneous detection of AME genes and to assess the frequency of these resistance genes in isolates of *E. coli* and *K. pneumoniae* in a single experiment.

MATERIALS AND METHODS

Bacterial sampling and culturing

A total of 95 bacterial strains (54 strains of *Klebsiella pneumoniae* and 41 strains of *E. coli* were isolated from urine sample (37), wound sample (28), throat swab samples (18) and sputum samples (12). They were collected, during the period of January, 2019 to December, 2020, from intensive care units in Mansoura University Hospital (MUH). These samples were handled in Microbiology Diagnostic and Infection Control Units (MDICU), Mansoura Faculty of Medicine for isolation and biochemical identification. Isolation was carried on blood agar and Macconkey agar and then incubated at 37^{o}C for 24 hours. For urine samples, inoculation on Cystine Lactose Electrolyte Deficient (CLED) Agar was carried out.

Bacterial identification

The obtained bacterial isolates were identified morphologically and biochemically according to the standard methods of Bergey's Manual of Determinative bacteriology (1985).

Antibiotics susceptibility testing

Antimicrobial susceptibility test was determined by disc diffusion method (Kirby-bauer) on Mueller Hinton agar (Oxoid, UK) using commercial antibiotic disks (Bioanalyse ASD, TURKEY) against gentamicin (10µg), amikacin (30µg), neomycin (30µg) and tobramycin (10µg). The results were interpreted according to Clinical and Laboratory Standards Institute Guideline (CLSI, 2020).

Detection of resistant genes

Extraction of bacterial DNA

Freshly grown bacterial colonies were collected separately from the culture plate of each isolate with a sterile bacteriological loop and suspended in 1 ml of sterile distilled water. The suspended bacterial solution was centrifuged for 10 minutes at 5000x g. The supernatant was discarded, and the pellet was used for DNA isolation. Bacterial genomic DNA was prepared using Genomic DNA Extraction Kit (RBC Bioscience, Taiwan) according to the manufacturer instructions.

Multiplex PCR application

The oligonucleotide primers, used for PCR amplification, were obtained from Biosearch technology CA USA (Table 1). No positive controls were used in all PCR reactions where the products of PCR were compared to the molecular size (bp) of targeting genes. For detection of Aminoglycosides resistance genes standard PCR technique was performed via by means of three specific sets of primers to *aph*(3), *ant*(2) and *aac*(6) as described by Kim *et al.*(2012). Reaction was done in a total volume of 25µl using 12.5µl of master mix (Bioline, UK), 5µl DNA template, 1µl of each upstream and downstream primers (10 pmol/ml) and volume was completed with 5.5 µl free RNA water. The thermal profile reaction was initial denaturation at 95°C for 5 mins; followed by 40 cycles of denaturation at 95°C for 30 s, annealing at 56°C for 1 minute and extension 72°C for 1 minute, followed by terminal extension at 72°C for 10 mins. Amplified DNA products were visualized on 2% agarose gels (Bio Basic INC, Canada) under the appropriate conditions, and then stained with ethidium bromide, and photographed using Canon Digital Camera under UV light.

Table (1): Nucleotide sequences of the primers used to amplify the aminoglycoside resistance genes.

Targeted Aminoglycoside resistance-gene	Primer sequence used	PCR Product size (bp)
aph(3)	5`-ATGGGCTCGCGATAATGTCG-3` 5`-AGAAAAACTCATCGAGCATC-3`	734
ant(2)	5`-ATGCAAGTAGCGTATGCGCT-3` 5`-TCCCCGATCTCCGCTAAGAA-3`	477
aac(6)	5`-AGTACTTGCCAAGCGTTTTAGCGC-3` 5`-CATGTACACGGCTGGACCAT-3`	365

RESULTS

Isolation and identification of isolates

From 250 samples, *Klebsiella pneumoniae and Escherichia coli* isolates were identified and confirmed biochemically. A total of 95 isolates were tested and found to be resistant to aminoglycoside antibiotics.

Antibiotic susceptibility testing

The data obtained from antibiotic susceptibility analysis are shown in Table (2). The *K. pneumoniae* isolates showed maximum resistance to neomycin (85.1%) followed by tobramycin (53.7%). Of the *K. pneumoniae* isolates, 44.4% were resistance to amikacin and 40.7% were resistance to gentamycin. However, the isolates of *E. coli* showed maximum resistance to neomycin (75.6%) followed by gentamicin (73.1%). Twenty two (53.6%) isolates were resistance to tobramycin, whereas only 13 isolates (31.7%) were resistance to amikacin. Meanwhile, isolates of both *K. pneumoniae* and *E. coli* showed a similar pattern of neomycin antibiotic resistance (Table 2). Antibiotic susceptibility tests also recorded variability in the proportions of microorganisms resistant to aminoglycosides, as shown in Table (3). The study

recognized ten different resistance patterns to aminoglycosides, of which five involved both *E. coli* and *K. pneumoniae*, three were specific to *K. pneumoniae*, and two were specific to *E. coli*. Testing isolates were also shown to be concurrently resistant to gentamicin, amikacin, tobramycin, and neomycin.Ten (18.5%) cases of *K. pneumoniae* and six (or 14.6%) cases of *E. coli* had the fourth resistant-pattern (IVa) responsive to all investigated AMGs except Neomycin resistance

Seven isolates of *K. pneumoniae* (12.9%) were found to exhibit the first pattern (I) resistance to the investigated AMGs, compared to five (12.2%) *E. coli* strains. Four of the *K. pneumoniae* strains (7.4%) and three (7.3%) of the *E. coli* strains displayed the resistance phenotype (IIb) to Gentamicin, Amikacin, and Neomycin. Nine strains of *K. pneumoniae* were found to be resistant to tobramycin and neomycin, whereas five isolates of *E. coli* were shown to have a third pattern of resistance (IIIa). The pattern (IIIb) was represented by 4 (7.4%) and 5 (12.2%) for *K. pneumoniae* and *E. coli*, respectively. It was resistant to Gentamicin and Amikacin and sensitive to Tobramycin and Neomycin. The pattern IVa, sensitive to all investigated AMGs except Neomycin, were reported for *K. pneumoniae*10 (18.5%) and 6 (14.6%) for *E. coli*. However, phenotypic resistance pattern IVb and IVc were only identified in isolates of *K. pneumoniae* and *E. coli*, respectively (Table 3).

Table (2): Antibiotic susceptibility % of isolates of *K. pneumoniae* and *E. coli* against chosen aminoglycoside antibiotics.

Antibiotic used	Bacterial isolates susceptibility (%)	
	K. pneumoniae	*E. coli*
Gentamicin	22 (40.7%)	30 (73.1%)
Amikacin	24 (44.4%)	13 (31.7%)
Tobramycin	29 (53.7%)	22 (53.6%)
Neomycin	46 (85.1%)	31 (75.6%)

Genotyping of the bacteriological isolates

The results of PCR as summarized in Table 4 revealed that, ninety-five isolates were utilised in the current study. We studied three aminoglycoside resistance genes that encode AMEs found in Gram-negative bacteria by designed three sets of primers that were specific for *aac*(6), *ant*(2) and *aph*(3) genes (see Table 1). To characterize the AME genes detected in *E. coli* and *K. pneumoniae*, the effect of primer concentration (5.0-15.0 pmol) and annealing temperature (53.0-58.0°C) were examined using multiplex PCR**.** The optimal primer concentration and annealing temperature for multiplex PCR detection of AME genes was found to be 10.0 pmol (each primer) and 56.0°C, respectively. Amplified DNA fragments of three different sizes (365, 477, and 734 bp) were able to detect up to 5-10 cell/ml (Fig. 1).

The PCR product patterns of representative strains showed the expected sizes of the amplified *aac*(6), *ant*(2) and *aph*(3) genes, in both isolate types, either as monoplex (lane 1-3) or multiplex (lane 4) as represented in Fig. (2). Non-specific background amplification products were not detected in this multiplex PCR assay. Therefore, the specificity of the primers selected in this study for multiplex-PCR was proved. The results also revealed that 8 out of 54 *K. pneumoniae* acquired *aac*(6) (14.81%) while *E. coli* isolates recorded 6 out of 41 *E. coli* (14.63%) acquired the same gene *aac*(6). The *ant*(2) gene was detected in 4 out of 54 *K. pneumoniae* (7.41%) and 6 out of 41 *E. coli* isolates (14.63%), while *aac*(6) and *ant*(2) genes were found together in 17 isolates, 12 from *K. pneumoniae* (22.22%) and five from *E. coli* (12.19). Detection of both *aac*(6) and *aph*(3) genes were found simultaneously only in eight *E.coli* isolates with frequency of 19.51%. Meanwhile, they did not detected in any *K. pneumoniae*. However, four isolates of the *K. pneumoniae* were found to contain both *ant*(2) and *aph*(3) genes with percentage of 7.41. In addition, eight out of 95 isolates of *E. coli* and *K. pneumoniae*, four isolates from each genus, were not found to have any AME genes (Table 4). The coexistence of three genes, *aac*(6), *ant*(2) and *aph*(3), was recognized in both *K. pneumoniae* and *E. coli* in 22(40.74%) 12(29.27), respectively (Fig. 3 and 4).

DISCUSSION

The increasing of multidrug-resistant species of *E. coli* and *K. pneumoniae* that produce aminoglycoside-modifying enzymes, extended-spectrum ß-lactamases (ESBLs) and AmpC enzymes has restricted options of treatment (Shahid *et al.*, 2003; Ananthakrishnan *et al*., 2000). Early diagnosis of infections caused by these organisms is therefore crucial, as prompt treatment may lower mortality in hospitalised patients (Rao and Shivanada, 1993; Veenu and Arora, 1998).

Aminoglycosides (AMGs) play an important role in serious *E. coli* and *K. pneumoniae* infections, despite reports of increased resistance to drugs. Several reports have stated that aminoglycoside (gentamicin) resistance is closely related to ciprofloxacin resistance (Haller, 1985; Mulder *et al*., 1997; Mandal *et al*., 2003; Pépin *et al*., 2009). Gentamicin was the most active against Gram-negative bacteria, including *E. coli and K. pneumoniae*, and is often used in combination with either ß-lactam or daptomycin (Leclercq *et al*., 1991; Moulds and Jeyasingham, 2010). The AMEs were classified as *aac*, *aph*, and *ant*. Many studies have found a correlation between common genes encoding for AMEs and aminoglycoside resistance (Kobayashi *et al*., 2001; Choi *et al*., 2003; Vakulenko *et al.*, 2003; Chen *et al*., 2005).

In this study, we investigated *aac*(6), *ant*(2) and *aph*(3) genes in clinical isolates of *E. coli* and *K. pneumoniae* that possessed high-level of resistance to gentamicin, amikacin, neomycin and tobramycin. In comparison to previous studies (Ghatole *et al*., 2004) that looked at *E. coli* and *K. pneumoniae* susceptibility to various antibiotics, this study found that *E. coli* and *K. pneumoniae* were more resistant to antibiotics as

Table (3): Phenotypic resistance patterns for isolates of *K. pneumoniae* and *E. coli*.

Bacterial isolates	Designated Pattern type	Number of isolates	Sensitivity of Antibiotic used†				Represented percentage (%)
			CN	AK	TOB	N	
K. pneumoniae	I	7	R	R	R	R	12.9
	IIa	9	S	R	R	R	16.7
	IIb	4	R	R	S	R	7.4
	IIIa	9	S	S	R	R	16.7
	IIIb	4	R	R	S	S	7.4
	IIIc	7	R	S	S	R	12.9
	IVa	10	S	S	S	R	18.5
	IVb	4	S	S	R	S	7.4
E. coli	I	5	R	R	R	R	12.2
	IIb	3	R	R	S	R	7.3
	IIc	12	R	S	R	R	29.3
	IIIa	5	S	S	R	R	12.2
	IIIb	5	R	R	S	S	12.2
	IVa	6	S	S	S	R	14.6
	IVc	5	R	S	S	S	12.2

†CN, Gentamicin; AK, Amikacin; TOB, Tobramycin; N, Neomycin; R, Resistant; S, Sensitive.

Table (4): Mono and Multi-Aminoglycoside Gene Resistance detected in *K.pneumoniae* and *E. coli* and their frequency.

Resistant Genes	No. of detected genes		Gene frequency (%)	
	K.pneumoniae	*E. coli*	*K.pneumoniae*	*E. coli*
***aac*(6) - *ant*(2) - *aph*(3)**	22	12	40.74	29.27
***aac*(6) - *ant*(2)**	12	5	22.22	12.19
***ant*(2) - *aph*(3)**	4	0	7.41	0
***aac*(6) - *aph*(3)**	0	8	0	19.51
***aac*(6)**	8	6	14.81	14.63
***ant*(2)**	4	6	7.41	14.63

there is a clear tendency towards decreased susceptibility for entirely groups of antibiotics. Additionally, in our study *K. pneumoniae* and *E. coli* isolates showed variable degrees of resistance against tested aminoglycosides as shown in Table 2. Among *K. pneumoniae* the highest percentages of resistance were observed for neomycin (85.1%) and tobramycin (53.7%) followed by amikacin (44.4%) and gentamycin (40.7%), while *E. coli*, the highest rates of resistance was observed for neomycin (75.6%) and gentamicin (73.1%) followed by tobramycin (53.6%) and amikacin (31.7%). Summarizing the results of susceptibility tests, neomycin was the aminoglycoside with the highest level of resistance against *K. pneumoniae* as well as *E. coli* with the percentages of susceptibility at 85.1% and 75.6%, respectively. The next highest antibiotic resistance was tobramycin (53.7%) for *K. pneumoniae*, while for *E. coli* was gentamycin (73.1%).

Resistance to aminoglycosides was also the subject of research performed by Lindemann *et al*. (2012) who tested ESBL-producing *E. coli* clinical isolates and revealed high rates of their resistance to gentamicin (80.6%), netilmicin (89.4%), and tobramycin (94%). Moreover, this work is in accordance with our study in the aspect of high activity of amikacin against ESBL producers (6% resistance). The low rates of reduced susceptibility against amikacin were also observed by Haldorsen *et al*. (2014) that reached the level of 0.4%, whereas for gentamicin and tobramycin these rates were 3.2% and 3.4%, respectively.

Regarding to PCR detection, generally, *aph*(3) gene out of other three AME-genes examined in current study have not been identified individually among *E. coli* and *K. pneumoniae* but coexist with another genes. Each isolate of *E. coli* and *K. pneumoniae* acquired one or more resistance genes. The most prevalent AME-genes among the *K. pneumoniae*, 54 isolates) were *aac*(6) and *ant*(2) (77.78%), followed by aph(3) (48.15%). While among the *E. coli* isolates the most prevalent AME-gene resistance was *aac*(6) (75.61%) followed by *ant*(2) (56.1%) and *aph*(3) (48.78%). Twenty-two (40.74%) isolate of *K. pneu-moniae* and twelve (29.27%) isolate of *E. coli*, each one of them acquired three AME-gene (*aac*(6), *ant*(2) and aph3). The PCR results revealed also that the coexists *aac*(6) and *ant*(2) have been detected among *K. pneumoniae* and *E. coli* with 22.22% and 12.19%, respectively.

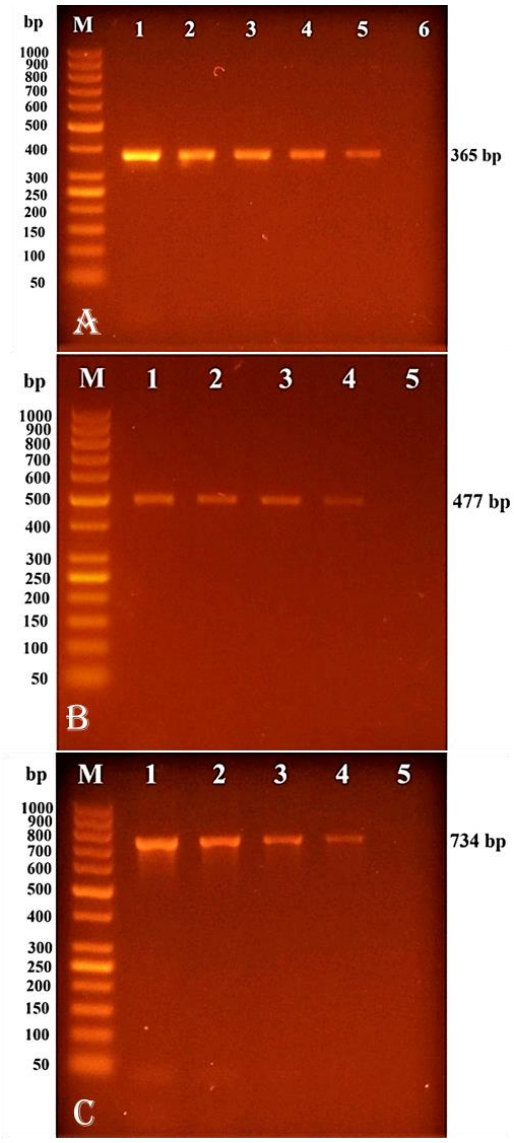

Figure (1): Electrophoresis Profile of the Polymerase Chain Reaction Products of Clinical *E. coli* and *K. pneumoniae* isolates containing Aminoglycoside Resistance Genes. Sensitivity detection for minimal cell concentration required to yield PCR products was about 5-10 cell/ml. A, *aph*(3), (lane 5); B, *ant*(2), (lane 4) and C, *aac*(6), (lane 4) genes. Lane M: 1000 base pair (bp) marker; lanes 1, 4 and 5: different cell concentrations (cell/ml) of the clinical isolates.

The *aac*(6) gene was most prevalent AMEs encountered in 77.7% in *K. pneumoniae* and 75.6% in *E.coli*, which is similar to those of other reports from India and abroad (Shahid and Malik, 2005; Ndegwa *et al.*, 2010; Moniri *et al*., 2010). It is noteworthy that *aac*(6) enzyme has got notable attention as to be implicated in the resistance of kanamycins and tobramycin as well as amikacin and neitlmicin (Schmitz *et al*., 1999).

In Poland PCR assays revealed the presence of *aac*(6)-Ib among 26 (59.2%) strains, *aph*(3)-Ib among 26 (59.2%) strains, *aph*(3)-Ib among 16 (36.2%), *aac*(3)-Ia among 7 (15.9%), and *ant*(2)-Ia among 2 (4.6%) strains, *aac*(6)-Ib and *aph*(3)- Ib genes were common among ESBL non-producers, and were detected among 4 (26,7%) and 3 (20%) strains, respectively; whereas, among ESBL producers, the most frequently detected genes encoding AMEs were *aac*(6)-Ib and *aph*(3)-Ib, observed in 22 (75.9%) and 13 (44.8%) of isolates, respectively. In addition, few ESBL-producing strains presented *aac*(3)-Ia and *ant*(2)-Ia genes. Additionally, they noticed that one isolates harbored three genes encoding AMEs: *aph*(3)-Ib, *aac*(3)-Ia, *aac*(6)-Ib (Ojdana *et al*., 2018). However, *the ant* (2) gene was observed 77.7% and 56.1% in *K. pneumoniae* and *E. coli* strains, respectively in which is in deferent with an earlier study from Iran in 250 isolates of *P. aeruginosa* obtained from various clinical specimens, which reported that *ant*(2) was prevalent detected in 28% of clinical isolates (Vaziri *et al*. 2011). Meanwhile, the data revealed that the least prevalence 48% of *aph*(3) which is similar to what has been observed in earlier study from India in *Enterococcus* species, reported a high prevalence (40.4%) of *aph*(3) (Padmasini *et al*. 2013).

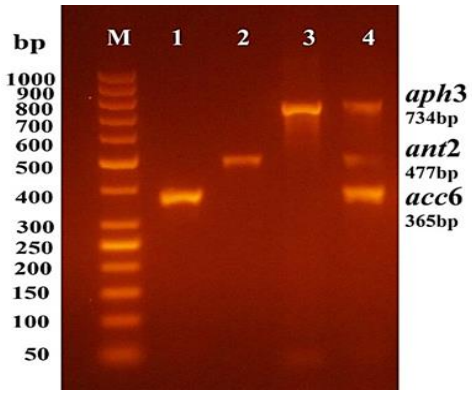

Figure 2. Individual monoplex (lane 1, 2 and 3) and multiplex (lane 4) PCR products for *aph*(3), *ant*(2) and aac6 genes, respectively. Lane M: 1000 base pair (bp) marker.

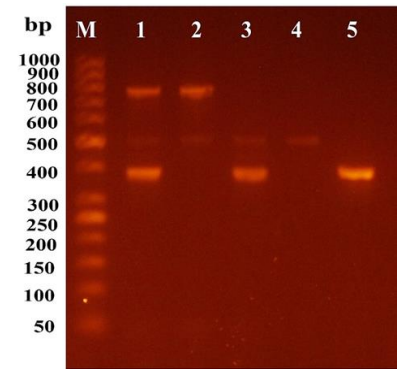

Figure (3): Multiplex PCR patterns for *K.pneumoniae* strains.

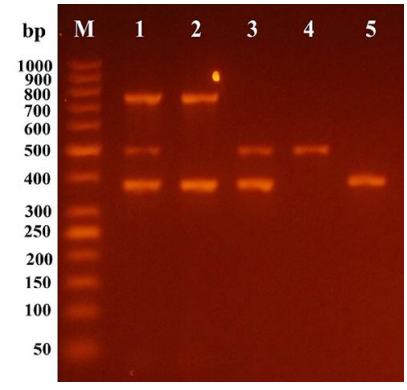

Figure (4): Multiplex PCR patterns for *E. coli* strains.

The results of multiplex-PCR revealed that each isolate of *E. coli* and *K. pneumoniae* acquired one or more resistance AMEs genes causing multidrug resistance problem. It was proven that the same gene of resistance was transferred horizontally between different bacterial species (Padmasini *et al.*, 2013).

CONCLUSION

A total of 95 clinical isolates have been isolated from Egyptian hospitals and laboratories (January 2019 to December 2020). Out of 95 bacterial isolates, 54 isolates were *K. pneumoniae* (56.8%) and 41 isolates were *E. coli* (43.2%). The most efficient amino-glycoside antibiotic among Egyptian clinical bacterial isolates was Amikacin. Aminoglycosides are used to treat both gram positive and gram-negative bacterial infections. This group of antibiotics is a second line drug which is prescribed in combination with other groups in clinical settings. Carriage of aminoglycoside resistance and their horizontal transferability in hospital setting demands urgent need to devise proper antibiotic policy and to slow down their expansion from hospital to community environment.

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الكشف عن الجينات المقاومة للأمينوجليكوسيد بواسطة تفاعل البوليميريز المتسلسل المتعدد لـلكلبسيلا الرئويه و الاشريشيا كولاى

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الملخص العربـــــي

تعتبر مجموعة الأمينوجليكوسيد هى فئة مهمة من المضادات الحيوية التى تستخدم غالبًا في علاج الالتهابات الشديدة التي تسببها البكتيريا سالبة الجرام وموجبة الجرام والتى لها القدره على إفرازإنزيم البيتا لاكتام مما يترتب عليه زيادة مقاومة هذه البكتريا تجاه المضادات الحيوية. من خلال هذه الدراسة تم فصل 95 عزلة من البكتريا عبارة عن 54 من الكليبسيلا الرئوية و 41 عزلة من الإشيريشيا كولاى وتم إجراء إختبار الحساسية لهذه البكتيريا تجاه عدد من المضادات الحيوية (الجنتاميسين ، الأميكاسين ، النيومايسين والتوبراميسين) باستخدام طريقة الانتشار القرصي (كيربي باور).وكذالك الكشف عن بعض الجينات المسؤلة عن مقاومة هذه البكتريا باستخدام تقنية تفاعل البلمرة المتسلسل المتعدد باستخدام ثلاث مجموعات محددة من البادئات للجينات(3) *aph* و و(2)*ant* و (6)*aac*. وقد أسفرت نتائج الحساسية لمضادات الميكروبات للسلالات المختبرة عن وجود عشرة أنماط مختلفة فى مقاومة الإشيريشيا كولاى والبكتريا الرئوية لهذه المضادات الحيوية، كما أظهرت نتائج تفاعل البلمرة المتسلسل المتعدد أن جينات الأمينوجليكوسيد هى الأكثر انتشارًا بين البكتريا الرئوية، حيث أظهرت البدئات للجينات (6)*aac* و(2)*ant* نسبة 77.78% ، تليها 48.15% (3) *aph* ، بينما عزلات الإشيريشيا كولاى كانت المقاومة الجينية الأكثر انتشارًا هي (6)*aac* 75.61% تليها (56.1%) (2)*ant* و (48.78%) (3) *aph*، كما أكدت النتائج أن كل عزلة قد اكتسبت جينًا مقاومًا واحدًا أو أكثر مما تسبب فى زيادة مقاومة البكتريا للمضادات الحيوية.