British Microbiology Research Journal
3(4): 678-691, 2013

SCIENCEDOMAIN *international*
www.sciencedomain.org

Aminoglycoside Resistance Patterns of Certain Gram Negative Uropathogens Recovered from Hospitalized Egyptian Patients

Samira M. Hamed[1], Khaled M. A. Aboshanab[2*], Walid F. Elkhatib[2], and Mohamed S. Ashour[1]

[1]Microbiology and Immunology department, Faculty of Pharmacy, Modern Sciences and Arts University, 6th of October, Egypt.
[2]Microbiology and Immunology department, Faculty of Pharmacy, Ain Shams University, Cairo, Egypt.

Authors' contributions

This work was carried out in collaboration between all authors. Author SMH performed the experiments, wrote the first draft of the manuscript, and searched the literature. Author KMAA designed the experiments, wrote the protocol, and managed the analysis of the study. Authors WFE and MSA managed the literature and analysis of study. All authors read and approved the final manuscript.

Research Article

Received 6th June 2013
Accepted 9th August 2013
Published 28th August 2013

ABSTRACT

Aims: To determine resistance rates and patterns of certain uropathogens, including *E. coli, Klebsiella spp.* and *Pseudomonas spp.,* isolated from hospitalized urinary tract infections patients, to aminoglycoside antibiotics and to detect the most prevalent plasmid-mediated aminoglycoside modifying enzymes (AMEs).
Methods: Uropathogenic isolates (150) were recovered from urine specimens of hospitalized UTI patients in Cairo, Egypt and identified by conventional methods. The recovered uropathogens *(E. coli, Klebsiella spp.* and *Pseudomonas spp.)* were tested for their susceptibility to gentamicin, tobramycin, amikacin, neomycin, netilmicin, and kanamycin by disc diffusion method. Plasmid-mediated aminoglycoside resistance was determined by transformation experiments as well as by using plasmids as templates for PCR screening of the AMEs-coding genes *aph(3')-I, aac(6')-I, aac(3)-I, aac(3)-II* and *ant(2")-I* in all resistant isolates.

**Corresponding author: Email: aboshanab2003@yahoo.com;*

Results: Of a total of 150 uropathogenic clinical isolates, 110 isolates were of the above mentioned genera and were selected for the current study. Sixty three isolates (57.2%) were resistant to at least one aminoglycoside antibiotic. Highest and lowest resistance rates were observed to kanamycin (53.6%) and amikacin (7.2%), respectively. The resistance rates to gentamicin, neomycin, tobramycin and netilmicin were 33.6%, 24.5%, 23.6% and 14.5%, respectively. AMEs-coding genes were detected on the plasmids of 93.6% of resistant isolates with prevalence rates of 53.9% for *ant(2'')-I*, 38% for both *aac(6')-I* and *aac(3)-II* and 33.3% for *aph(3')-I*, while *aac(3)-I* gene was not detected in any of the tested resistant isolates. Double and triple combinations of AMEs-coding genes were detected in ich49.2% of resistant isolates.

Conclusion: A high prevalence of plasmid-mediated resistance to aminoglycoside antibiotics in Gram negative uropathogens from hospitalized patients was observed. Uropathogens may represent potential reservoirs of panaminoglycoside resistance in hospitals, having on their plasmids combinations of AMEs-coding genes. Good infection control measures in Egyptian hospitals together with periodic screening of prevalence rates of different resistance genes are required.

Keywords: Aminoglycoside antibiotics; Interpretative reading; Uropathogens; aph(3')-I; aac(6')-I; aac(3)-II; ant(2'')-I.

1. INTRODUCTION

In an era of rapid spread of bacterial resistance to many of antibiotics and decelerated discovery of new antimicrobial agents, the focus has returned to the re-evaluation of the use of old antibiotic compounds. Because of their limited use for years, being displaced by more active and less toxic agents, they may retain activity against a large number of currently prevalent resistant bacterial isolates. Aminoglycoside antibiotics are re-emerging as valuable alternatives for the treatment of difficult-to-treat infections, particularly those caused by Gram negative pathogens [1,2].

Aminoglycosides are a group of clinically important, broad-spectrum antibiotics that inhibit protein biosynthesis in bacteria by selectively binding to the A-site decoding region of the bacterial 16S rRNA within the 30S ribosomal subunit causing mistranslation of mRNA or premature termination of protein synthesis [3]. Advantages for aminoglycosides clinical use include: having the properties of bactericidal activity, predictable pharmacokinetics, synergy with other antibiotics and low cost. A particular advantage for urinary tract infections (UTIs) treatment is their ability to attain urine concentrations of 25-100 times that in serum [4,5]. Concentration-dependant bactericidal activity and postantibiotic effect allowed the once-daily dosing regimen which markedly decreased toxicities that limited their wide use for years [6]. Aminoglycoside monotherapy was found to produce equal efficacy with β-lactam drugs and fluoroquinolones in treatment of UTIs and pyelonephritis [7].

Since introduction into clinical use, three mechanisms are known to be responsible for bacterial resistance to aminoglycoside antibiotics [8]: first, decreased intracellular accumulation by outer membrane permeability alteration [9], diminished inner membrane transport [10], or active efflux [11-13]; second, target modification by mutation of 16S rRNA [14,15] or ribosomal proteins coding genes [16] or by 16S rRNA methylation, a mechanism newly identified in clinical isolates [17,18]; and third, enzyme-mediated drug modification resulting in compromised binding to target site, the most prevalent in clinical setting [19]. There are three classes of aminoglycoside modifying enzymes: aminoglycoside

phosphotransferases (APHs), aminoglycoside nucleotidyltransferases (ANTs), and aminoglycoside acetyltransferases (AACs). Within each class, there are enzymes with different regiospecificities for aminoglycosides modification [5].

Testing the resistance profile to a large panel of antibiotics has been long known to help in the prediction of the underlying resistance mechanism in bacterial isolates using "The interpretative reading" described before by Livermore *et al.* [20] but due to the recent increase in the complexity of the resistance phenotypes, molecular techniques must be used for identification of the exact resistance mechanism [21,22]. The current study has aimed at determining the resistance rates and patterns of certain uropathogens to different aminoglycoside antibiotics as well as detection of the most prevalent plasmid-mediated aminoglycoside modifying enzymes by phenotypic and genotypic methods.

2. MATERIALS AND METHODS

2.1 Collection, Identification and Storage of Bacterial Strains

A total of 150 bacterial isolates were recovered from urine specimens of UTI patients from four different hospitals in Cairo and were identified by conventional microbiological methods. Isolates of the genera *Escherichia, Klebsiella* and *Pseudomonas* were selected for this study whose total number was 110 isolates, and they have been stored as glycerol stock at -20ºC throughout the study.

2.2 Antimicrobial Susceptibility Testing

Susceptibility of the selected isolates to six aminoglycoside antibiotics was tested using commercial discs (Oxoid, UK) of the following antibiotics (µg/disc): gentamicin (10), tobramycin (10), amikacin (30), neomycin (30), netilmicin (30) and kanamycin (30) by disc diffusion method on Mueller Hinton agar according to Clinical and Laboratory Standards Institute (CLSI) guidelines [23]. Isolates were categorized as susceptible (S), intermediate (I) and resistant (R) depending on the measured inhibition zone diameters. Isolates showing resistant and intermediate resistance phenotypes were selected for polymerase chain reaction (PCR) assays of AMEs-coding genes. Interpretative reading of the resistance profile was used for prediction of the underlying resistance mechanism [20].

2.3 Plasmid Extraction

Plasmids were extracted from isolates having reduced susceptibility to at least one of the tested aminoglycoside antibiotics using GeneJet Plasmid Miniprep Kit (thermoscientific) according to the manufacturer's instructions. Presence, number and molecular sizes of plasmids in each isolate were detected by gel electrophoresis using GelPilot 1 kb DNA ladder (Qiagen, Germany).

2.4 Transformation Assay

Plasmid localization of resistance genes and their ability to transfer was tested by transformation experiments using *E. coli* DH5α as a recipient strain. Extracted plasmids from resistant isolates were used to transform chemically competent *E. coli* DH5α, prepared according the method described previously [24]. Successful transformation was confirmed by antibiogram analysis and PCR assays.

2.5 PCR Amplification of Genes Encoding Aminoglycoside Modifying Enzymes

Five AMEs-coding genes, *aac(6')-I*, *aph(3')-I, aac(3)-I, aac(3)-II and ant(2'')-I,* were selected for screening in 63 resistant isolates showing reduced susceptibility to at least one of the tested aminoglycoside antibiotics based on interpretative reading of the most commonly encountered resistance phenotypes [20] and dissemination in Gram negative isolates as reported in literature [19]. Five sets of primers were designed from sequences deposited in the GenBank database (Table 1). PCR assays were carried out in a final volume of 25 µl, consisting of 12.5 µl of 2X DreamTaq™ Green PCR Master Mix (Fermentas, USA), 1 µl (25 picomole) of each primer (Fermentas, USA) and 1µl of plasmid DNA and the reaction mixture was completed to 25 µl with DNA grade water, using various amplification conditions for each primer set. PCR products were detected by electrophoresis on 1% w/v agarose gel containing 0.5 µg/ml ethidium bromide and visualized on UV transilluminator. PCR products sizes were determined using ready-to-use GeneRuler™ 100bp Plus DNA Ladder (Fermentas, USA).

Table 1. Primer sequences and expected amplicon sizes for PCR products

Primer[a]	Primer sequence (5'→3')	Target genes (alternative names) (accession number)[b]	Amplicon Size (bp)
APH(3`)I-f	TTATGCCTCTTCCGACCATC	*aph(3')-Ia (aphA-1)* (V00359) *aph(3')-Ic (apha1-1AB, apha7)* (M37910)	223
APH(3`)I-r	GCCTGAGCGAGACGAAATAC		
AAC(6`)I-f	TTGCGATGCTCTATGAGTGG	*aac(6')-Ib (aacA4)* (M21682)	356
AAC(6`)I-r	CGTTTGGATCTTGGTGACCT		
AAC(3)I-f	CCACCTACTCCCAACATCA	*aac(3)-I (aacC1)* (X15852)	329
AAC(3)I-r	TTCCCGTATGCCCAACTTT		
AAC(3)II-f	GCAGAAGGCAATAACGGAG	*aac(3)-IIa (aaC3)* (X13543) *aac(3)-IIc (aacC2)* (X54723)	567
AAC(3)II-r	CCAGGCATCGGCATCTCATA		
ANT(2``)I-f	AAGCACGATGATATTGATCTG	*ant(2'')-Ia (aadB)* (X04555)	288
ANT(2``)I-r	GGCATAGTAAAAGTAATCCCA		

[a] *f, forward primer; r, reverse primer*

[b] *Alternative names and accession numbers of target genes as described by Ramirez et al. [19]*

2. 6. DNA Sequencing

Four amplicons representative of the positive four sets of primers were verified by sequencing on both strands (Macrogen, Korea). The nucleotide sequences of the amplicons were analyzed using the open access software of National Center of Biotechnology Information web site (http://www.ncbi.nlm.nih.gov).

3. RESULTS

3.1 Isolates Identification and Aminoglycoside Resistance Profile

Among 110 isolates, 74(67.2%), 24(21.8%) and 12(10.9%) isolates were identified to be *Escherichia coli*, *Klebsiella spp.* and *Pseudomonas spp.,* respectively and these isolates were selected for further studies. Regarding the resistance profile of the isolates, 63(57.2%) isolates had shown resistance to at least one of the six tested aminoglycoside antibiotics. The resistance distribution among the genera was as follows: *Escherichia coli* (64.0%), *Klebsiella spp.* (15.6%) and *Pseudomonas spp*. (18.7%). Regardless of the genera, the highest resistance rate was to kanamycin (53.6%) and the lowest resistance rate was to amikacin (7.2%). Resistance rates to gentamicin, neomycin, tobramycin and netilmicin were 33.6%, 24.5%, 23.6% and 14.5%, respectively. Resistance rates to aminoglycoside antibiotics differed markedly in isolates of different genera as shown in Table (2).

Table 2. Resistance rates of different isolates, including *Escherichia coli, Klebsiella spp.* and *Pseudomonas spp.* to the tested aminoglycoside antibiotics

Genus	No. of isolates resistant to each aminoglycoside antibiotic[a,b]					
	G	T	A	Nm	Nt	K
Escherichia coli (n=74)	21(28.3%)	12(16.2%)	3(4.0)%	23(31.0%)	5(6.7%)	38(51.3%)
Klebsiella spp. (n=24)	8(33.3%)	5(20.8%)	1(4.1%)	1(4.1%)	3(12.5%)	9(37.5%)
Pseudomonas spp. (n=12)	8(66.6%)	9(75.0%)	4(33.3%)	3(25.0%)	8(66.6%)	12(100.0%)
Total (n=110)	37(33.6%)	26(23.6%)	8(7.2%)	27(24.5%)	16(14.5%)	59(53.6%)

[a]*G, gentamicin; T, tobramycin; A, amikacin; Nm, neomycin; Nt, netilmicin; K, kanamycin*
[b]*Percentage of resistance to each antibiotic was calculated as compared to the number of isolates in each genus*

3.2 Transformation

Transformation experiments were successful in only seven isolates (11.1%) out of 63 resistant ones. The transformation was confirmed by antimicrobial susceptibility testing and PCR assay (Table 3; Fig. 1). The resistance profile was identical in transformants and donor clinical isolates except in one transformant (116^T) where resistance to gentamicin was lost, as compared to the donor clinical isolate. *ant(2'')-I* gene was not detected in this transformant (116^T) despite being detected in the donor isolate (116).

Table 3. Resistance profile and genes detected in donor clinical isolates and transformants

Isolate No.	Donor Clinical Isolate		Transformant	
	Resistance Profile	Genes detected	Resistance Profile	Genes detected
32	Nm^R-K^R	*aph(3')-I*	Nm^R-K^R	*aph(3')-I*
70	Nm^R-K^R	*aph(3')-I*	Nm^R-K^R	*aph(3')-I*
77	Nm^R-K^R	*aph(3')-I*	Nm^R-K^R	*aph(3')-I*
98	T^R-K^R	*ant(2")-I*	T^R-K^R	*ant(2")-I*
116	G^R-Nm^R-K^R	*aph(3')-I, ant(2")-I*	Nm^R-K^R	*aph(3')-I*
164	G^R-T^R-K^R	*ant(2")-I*	G^R-T^R-K^R	*ant(2")-I*
152	G^R-Nm^R-K^R	*aph(3')-I, ant(2")-I, aac(3)-II*	G^R-Nm^R-K^R	*aph(3')-I, ant(2")-I, aac(3)-II*

**G^R, resistant to gentamicin; T^R, resistant to tobramycin; Nm^R, resistant to neomycin; K^R, resistant to kanamycin*

3.3 Prevalence of AMEs-coding Genes in *E. coli, Klebsiella spp.* and *Pseudomonas spp.* Isolates

Among 63 tested isolates, 59(93.6%) were found to carry AMEs-coding genes on their plasmids. *ant(2")-I* was the most frequently encountered gene (53.9%) followed by *aac(6')-I* and *aac(3)-II* each were found in 24 isolates (38%). Twenty-one isolates (33.3%) were found to carry *aph(3')-I,* while *aac(3)-I* gene was not detected in any of the tested resistant isolates. Regarding the distribution of the four AMEs-coding genes in resistant isolates of different genera, *ant(2")-I* was detected in 46.3% of *E.coli* resistant isolates, *aph(3')-I* in 44,1% , while 34.1% and 31.5% of them carried *aac(3)-II* and *aac(6')-I*, respectively. In resistant *Klebsiella spp.* isolates, *ant(2")-I* was the most commonly detected (70.0%), followed by *aac(3)-II* (60.0%), *aac(6')-I* (40.0%), then *aph(3')-I* was the least commonly detected in 20.0% of *Klebsiella spp.* resistant isolates. The prevalence rates of the four AMEs genes in resistant *Pseudomonas spp.* was 66.6%, 58.3, 33.3% and 16.6% for *ant(2")-I*, *aac(6')-I*, *aac(3)-II* and *aph(3')-I*, respectively (Fig. 2).

Twenty-eight isolates (44.4%) had only one AME-coding gene, with *aph(3')-I* being the most common single one in twelve isolates (19.0%), while 31 isolates (49.2%) had more than one AMEs-coding genes. Coexistence of two and three AMEs-coding genes where detected in 18 isolates (28.5%) and 13 isolates (20.6%), respectively, having a total of nine different AMEs-coding genes combinations. The aforementioned genes could not be detected on the plasmids of only four resistant isolates (6.3%) despite showing resistance to at least one of the tested aminoglycoside antibiotics.

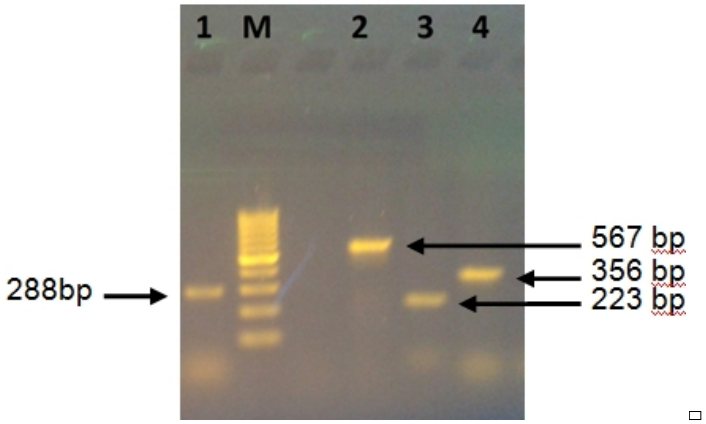

Fig. 1. Agarose gel electrophoresis of the amplification products of the four positive AMEs-coding genes: lane 1, *ant(2")-I* gene (288bp); lane M, 100bp ladder; lane 2, *aac(3)-II* gene (567bp); lane 3, *aph(3')-I* (223bp); lane 4, *aac(6')-I* (356bp)

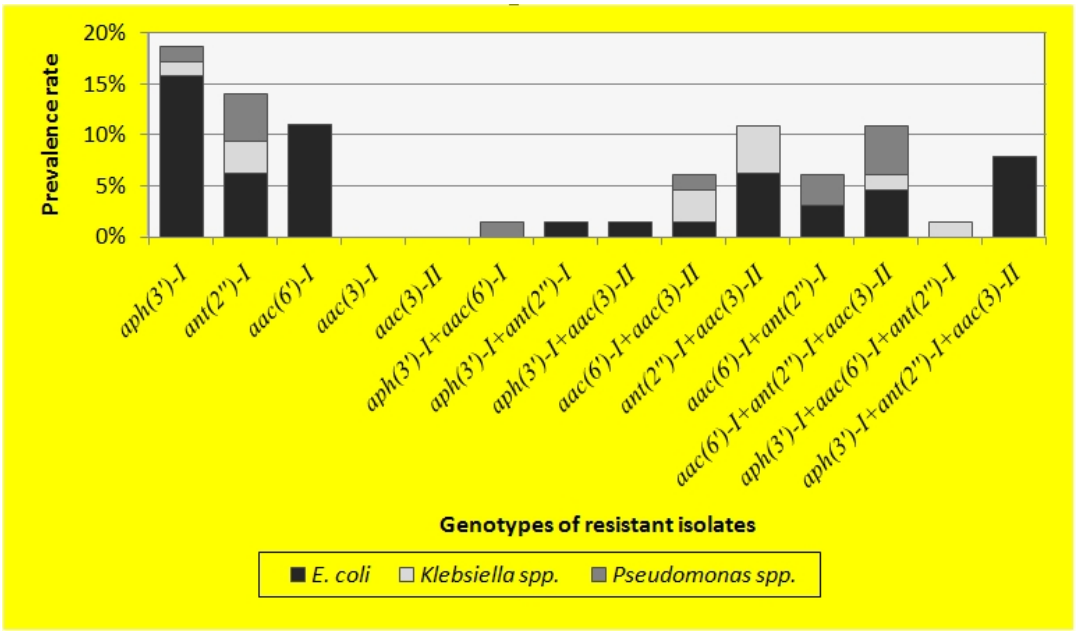

Fig. 2. Prevalence rates of different genotypes in resistant isolates of different genera
Percentage of each genotype is calculated as compared to the number of resistant isolates

3.4 Correlation between AMEs-coding Genes and Resistance to Aminglycoside Antibiotics

Resistance to aminoglycoside antibiotics matched the underlying genotype in 53 isolates (84.1%) for gentamicin, 43 isolates (68.2%) for tobramycin, 40 isolates (63.4%) for amikacin, 53 isolates (84.1%) for neomycin, 38 isolates (60.3%) for netilmicin and 59 isolates (93.6%) for kanamycin. Susceptibility to some aminglycoside antibiotics was retained despite

harboring resistance genes in five isolates (7.9%) for gentamicin, twenty isolates (31.7%) for tobramycin, eighteen isolates (28.5%) for amikacin, two isolates (3.1%) for neomycin, 23 isolates (36.5%) for netilmicin and two isolates (3.1%) for kanamycin. In only 11 isolates (17.4% of all resistant isolates), there was 100% concordance between genotype and resistance to all aminoglycoside antibiotics. Frequency of AMEs genotypes and observed phenotypes in comparison to expected resistance phenotypes as described before by Ramirez *et al*. [19] were listed in Table (4).

Table 4. Frequency of AMEs genotypes, expected resistance phenotypes versus observed phenotypes in tested isolates

AMEs coding genes	Number of isolates n=63(%)	Expected resistance phenotypes[a,b]	Observed resistance phenotypes (no. of isolates)
aph(3')-I	12(19.0%)	Nm^R-K^R	As expected(8) G^R-Nm^R-K^R (2) K^R(2)
aac(6')-I	7(11.1%)	A^R-T^R-Nt^R-K^R	As expected(0) T^R-K^R(3) K^R(3) A^R-K^R(1)
ant(2'')-I	9(14.2%)	G^R-T^R-K^R	As expected(1) G^R-A^R-T^R-Nt^R-K^R(1) G^R-T^R-Nm^R-K^R(1) T^R-K^R(2) K^R(2) G^R(1) T^R-Nt^R-K^R(1)
aac(3)-I	0(0.0%)	G^R	As expected(0)
aac(3)-II	0(0.0%)	G^R-T^R-Nt^R	As expected(0)
aph(3')-I+aac(6')-I	1(1.5%)	A^R-T^R-Nt^R-Nm^R-K^R	As expected(0) G^R-A^R-T^R-Nt^R-Nm^R-K^R(1)
aph(3')-I+ant(2'')-I	1(1.5%)	G^R-T^R-Nm^R-K^R	As expected(0) G^R-N^R-K^R(1)
aph(3')-I+aac(3)-II	1(1.5%)	G^R-Nm^R-K^R	As expected(1)
aac(6')-I+aac(3)-II	4(6.3%)	G^R-A^R-T^R-Nt^R-K^R	As expected(0) G^R-T^R-Nt^R-K^R(1) G^R-T^R-K^R(2) G^R-Nt^R-K^R(1)
ant(2'')-I+aac(3)-II	7(11.1%)	G^R-T^R-Nt^R-K^R	As expected(1) G^R-A^R(1) G^R-K^R(4) G^R-Nt^R-Nm^R-K^R(1)
aac(6')-I+ant(2'')-I	4(6.3%)	G^R-A^R-T^R-Nt^R-K^R	As expected(0) G^R-A^R-T^R-Nt^R-Nm^R-K^R(1) G^R-T^R-K^R(1) G^R-T^R-Nt^R-K^R(2)
aac(6')-I+ant(2'')-I+aac(3)-II	7(11.1%)	G^R-A^R-T^R-Nt^R-K^R	As expected(0) G^R-T^R-K^R(2) G^R-T^R-Nt^R-K^R(3) G^R-A^R-T^R-Nt^R-Nm^R-K^R(2)

aph(3')-I+aac(6')-I+ant(2")-I	1(1.5%)	G^R-A^R-T^R-Nt^R-Nm^R-K^R	As expected(1)
aph(3')-I+ant(2")-I+aac(3)-II	5(7.9%)	G^R-T^R-Nt^R-Nm^R-K^R	As expected(0) G^R-Nm^R-K^R(5)

[a] *G^R, resistant to gentamicin; T^R, resistant to tobramycin; A^R, resistant to amikacin; Nm^R, resistant to neomycin; Nt^R, resistant to netilmicin; K^R, resistant to kanamycin*
[b] *Expected phenotypes as described by Ramirez et al [19]*

4. DISCUSSION

Urinary tract infections (UTIs) are one of the most common bacterial infections affecting all age groups either in community or healthcare setting [25]. A limited geographical variability of uropathogen occurrence has been demonstrated by different studies being dominated by Gram negative pathogens, most commonly *Escherichia coli, Klebsiella spp.* and *Pseudomonas spp*. in hospitalized patients [26]. Other than representing a therapeutic threat to critically ill UTI patients and those in long-term care facilities, resistant uropathogens may become potential reservoirs of certain resistance phenotypes among hospitalized patients [26].

Several previous studies carried out in Egypt and different countries worldwide have examined the prevalence and mechanisms of aminoglycoside resistance in Gram negative uropathogens. In the current study, a high overall resistance rate (57.2%) to aminoglycoside antibiotics was found in tested isolates. The highest resistance rate was to kanamycin (53.6%) and the lowest was to amikacin (7.2%).

A previous study carried out in Upper Egypt in 2011 by Gad *et al.* [27] has reported higher resistance rates to gentamicin (36.0%), tobramycin (30.0%), amikacin (16.0%) and neomycin (48.0%), and lower resistance rate to kanamycin (44.0%) in *E. coli* isolates. In the same study, *Klebsiella spp.* isolates have shown higher resistance rates to gentamicin (62.5%), tobramycin (50%), amikacin (25%), neomycin (62.5%) and kanamycin (75%) than those of the current study. On the other hand, resistance rates of *E. coli* isolates to amikacin, gentamicin and tobramycin in the current study were higher than those detected in each of North America, Latin America and Asia-West Pacific area as reported by the SENTRY antimicrobial surveillance program that has monitored the antimicrobial resistance among uropathogens worldwide over a 4-year period (1997-2000). The same program has also reported lower resistance rates of *Klebsiella spp.* isolates to the same antibiotics in North America and Asia-West Pacific area and higher resistance rates in Latin America compared to our study [26,28,29].

For *Pseudomonas spp.* isolates, resistance rates found in the current study were higher than those reported in upper Egypt by Gad *et al.* [27] who have detected lower resistance rates to kanamycin (80.0%), gentamicin (55.6%), tobramycin (22.2%), and amikacin (20.0%) but higher resistance rate to neomycin (77.8%). In comparison to other studies conducted worldwide, resistance of *Pseudomonas spp.* isolates to amikacin, gentamicin, and tobramycin in this study were higher than those in Europe [30], Asia-West Pacific area [26], and North America [28] but in Latin America, *Pseudomonas spp.* isolates had shown lower resistance rates to both gentamicin (57.6%) and tobramycin (54.5%) and higher resistance rate to amikacin (51.5%) compared to our findings [29,31].

Screening of the resistant isolates for the presence of five AMEs-coding genes, known to be widely distributed among Gram negative bacilli by PCR assays has shown that, 44.4% of the

tested isolates had a single AME-coding gene while the majority (55.6%) had a combination of two or three. Out of five tested AMEs-coding genes, *ant(2'')-I* was the most prevalent (53.9%) either individual, in 14.5%, or combined with other AMEs-coding genes in 39.6% of resistant isolates, followed by both *aac(6')-I* and *aac(3)-II* each detected in 38.0% of resistant isolates, *aac(6')-I* was detected as an individual resistant mechanism in 11.1% of resistant isolates and combined to other AMEs genes in 26.9% of them, while *aac(3)-II* existed in combination to other AMEs-coding genes in all of its positive isolates.

Regarding the order of prevalence of the four detected genes in the three genera, *ant(2'')-I* was the most prevalent followed by *aph(3')-I*, *aac(3)-II*, then *aac(6')-I* in *E. coli* isolates, *aac(3)-II*, *aac(6')-I*, then *aph(3')-I* in *Klebsiella* spp isolates and *aac(6')-I*, *aac(3)-II,* then *aph(3')-I* in *Pseudomonas spp.*. Recent studies have reported the predominance of *aac(6')-I* in France [32] and *aac(6')-II* in Iran [33] in *Pseudomonas spp.* isolates.

In the current study, despite being the least prevalent, *aph(3')-I* was the most frequent individual AME-coding gene in *E. coli* isolates, this is in line with the findings of Gad *et al.* [27] although it was reported as a rare mechanism among Gram negative isolates studied by Over *et al.* in Turkey [34]. *ant(2'')-I* was the most common individual AME-coding gene detected in *Klebsiella spp.* and *Pseudomonas.spp.* isolates Different studies around the world in the period from 1988 to 1993 have reported *ant(2'')-I* as the most common individual resistance mechanism to aminoglycoside antibiotics among *Enterobacteriaceae* isolates in Turkey [34,35], USA, and Japan and *aac(3)-II* in Europe, Latin America and South Africa [36,37]. In the same time period, the most prevalent individual resistance mechanisms detected in *Pseudomonas spp.* isolates were *ant(2'')-I* in USA and *aac(6')-II* in Europe, Latin America, South Africa and Japan.

Nine different combinations of AMEs were encountered in the current study of which both [*aac(6')-I+ant(2'')-I+aac(3)-II*] and [*ant(2'')-I+aac(3)-II*] were the most common combinations equally detected in 11.1% of all resistant isolates, followed by the combination [*aph(3')-I+ant(2'')-I+aac(3)-II*]. The two combinations [*aac(6')-I+aac(3)-II*] and [*aac(6')-I+ant(2'')-I*] were equally existent in 6.3% of isolates. Other rare combinations each detected in 1.5% of resistant isolates include [*aph(3')-I+aac(6')-I+ant(2'')-I*], [*aph(3')-I+ant(2'')-I*], [*aph(3')-I+ant(2'')-I*] and [*aph(3')-I+aac(6')-I*]. Of all combinations detected, those of *aac(6')-I* and gentamicin-modifying enzymes coding genes (*ant(2'')-I* and *aac(3)-II*) are the most important [36]. With the existence of such combinations on the plasmids of 25.3% of resistant isolates, dissemination of panaminoglycoside resistance among clinical isolates becomes a potential threat.

A notable finding, revealed by this study, was the poor correlation between genotypes and resistance phenotypes with 100% concordance in only 17.4% of resistant isolates, this was accompanied by retained susceptibility to one or more antibiotics despite existence of AMEs-coding genes to which they are known to be substrates. Only 75.0% of *aac(6')-I* gene-carriers were resistant to amikacin, similar findings were reported in previous studies [38,39]. Despite being a substrate of enzymes coded by both *ant(2'')-I* and *aac(3)-II,* gentamicin retained activity against 14.7% of ANT(2'')-I producers while none of *aac(3)-II* carriers were sensitive to gentamicin. Similarly, tobramycin remained active against 20.8%, 44.1%, and 54.1% of *aac(6')-I*, *ant(2'')-I*, and *aac(3)-II* carrier isolates, respectively. 50.0% of *aac(6')-I* carriers and 62.5% of *aac(3)-II*-carriers have lost their ability to inactivate netilmicin. On the other hand, there was a conserved ability to modify kanamycin in 100% of isolates carrying either *aph(3')-I* or *aac(6')-Ib*, while 5.8% of *ant(2'')-I* carriers could not inactivate kanamycin. Based on the above mentioned findings, the loss of the potential of some AMEs to modify

certain aminoglycoside antibiotics is responsible for the difference in resistance rates to aminoglycosides in isolates harboring the same resistance AMEs-coding genes and consequently the overall resistance to different aminoglycosides.

Low success rate (11.1%) of plasmid-localization of resistance genes by phenotypic transformation experiments, despite detection by PCR assay on plasmids of resistant isolates, may be due to the following reasons: the dramatic reduction in transformation frequencies with larger plasmids (>15 kb) using heat shock-mediated transformation [40]; plasmid-mediated resistance genes may be carried by a plasmid other than that succeeded to transform *E. coli* DH5α; and the last reason, based on the finding that all successful transformations were achieved in *E. coli* donor isolates, is the failure of expression of acquired plasmids of *Klebsiella* and *Pseudomonas*-origin in *E. coli* DH5α host strain.

5. CONCLUSION

This study provided information about resistance to aminoglycoside antibiotics and most common resistance determinants providing useful comparative data for future studies. High prevalence of plasmid-mediated aminoglycoside resistance in uropathogens particularly *Pseudomonas spp.* isolates was observed. Combinations of different AMEs-coding genes were highly disseminated in resistant isolates most importantly amikacin- and gentamicin-modifying enzymes coding genes. Proper infection control measures and periodic monitoring of resistance to aminoglycoside antibiotics, using interpretative reading guided molecular techniques are required to minimize further dissemination and preserve the usefulness of this important class of antibiotics for treatment of complicated infections.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Falagas ME, Grammatikos AP, Michalopoulos A. Potential of old-generation antibiotics to address current need for new antibiotics. Expert Rev Anti Infect Ther. 2008;6(5):593-600.
2. Becker B, Cooper MA. Aminoglycoside Antibiotics in the 21st Century. ACS Chem Biol. 2012. 10.1021/cb3005116
3. Yang L, Ye XS. Development of aminoglycoside antibiotics effective against resistant bacterial strains. Curr Top Med Chem. 2010;10(18):1898-26.
4. Durante-Mangoni E, Grammatikos A, Utili R, Falagas ME. Do we still need the aminoglycosides? Int J Antimicrob Agents. 2009;33(3):201-5.
5. Kotra LP, Haddad J, Mobashery S. Aminoglycosides: perspectives on mechanisms of action and resistance and strategies to counter resistance. Antimicrob Agents Chemother. 2000;44(12):3249-56.
6. Vakulenko SB, Mobashery S. Versatility of aminoglycosides and prospects for their future. Clin Microbiol Rev. 2003;16(3):430-50.
7. Vidal L, Gafter-Gvili A, Borok S, Fraser A, Leibovici L, Paul M. Efficacy and safety of aminoglycoside monotherapy: systematic review and meta-analysis of randomized controlled trials. J Antimicrob Chemother. 2007;60(2):247-57.

8. Shaw KJ, Rather PN, Hare RS, Miller GH. Molecular genetics of aminoglycoside resistance genes and familial relationships of the aminoglycoside-modifying enzymes. Microbiol Rev. 1993;57(1):138-63.
9. Hancock RE. Alterations in outer membrane permeability. Annu Rev Microbiol. 1984;38:237-264.
10. Bryan LE, Kowand SK, Van Den Elzen HM. Mechanism of aminoglycoside antibiotic resistance in anaerobic bacteria: *Clostridium perfringens* and *Bacteroides fragilis*. Antimicrob Agents Chemother. 1979;15(1):7-13.
11. Magnet S, Courvalin P, Lambert T. Resistance-nodulation-cell division-type efflux pump involved in aminoglycoside resistance in *Acinetobacter baumannii* strain BM4454. Antimicrob Agents Chemother. 2001;45(12):3375-80.
12. Moore RA, DeShazer D, Reckseidler S, Weissman A, Woods DE. Efflux-mediated aminoglycoside and macrolide resistance in *Burkholderia pseudomallei*. Antimicrob Agents Chemother. 1999;43(3):465-70.
13. Poole K. Efflux-mediated multiresistance in Gram-negative bacteria. Clin Microbiol Infect. 2004;10(1):12-26.
14. Prammananan T, Sander P, Brown BA, Frischkorn K, Onyi GO, Zhang Y, *et al*. A single 16S ribosomal RNA substitution is responsible for resistance to amikacin and other 2-deoxystreptamine aminoglycosides in *Mycobacterium abscessus* and *Mycobacterium chelonae*. J Infect Dis. 1998;177(6):1573-81.
15. Recht MI, Douthwaite S, Dahlquist KD, Puglisi JD. Effect of mutations in the A site of 16 S rRNA on aminoglycoside antibiotic-ribosome interaction. J Mol Biol. 1999;286(1):33-43.
16. Basso LA, Blanchard JS. Resistance to antitubercular drugs. Adv Exp Med Biol. 1998;456:115-44.
17. Galimand M, Courvalin P, Lambert T. Plasmid-mediated high-level resistance to aminoglycosides in *Enterobacteriaceae* due to 16S rRNA methylation. Antimicrob Agents Chemother. 2003;47(8):2565-71.
18. Doi Y, Arakawa Y. 16S ribosomal RNA methylation: emerging resistance mechanism against aminoglycosides. Clin Infect Dis. 2007;45(1):88-94.
19. Ramirez MS, Tolmasky ME. Aminoglycoside modifying enzymes. Drug Resist Updat. 2010;13(6):151-71.
20. Livermore DM, Winstanley TG, Shannon KP. Interpretative reading: recognizing the unusual and inferring resistance mechanisms from resistance phenotypes. J Antimicrob Chemother. 2001;48 Suppl 1:87-102.
21. Flamm RK, Phillips KL, Tenover FC, Plorde JJ. A survey of clinical isolates of *Enterobacteriaceae* using a series of DNA probes for aminoglycoside resistance genes. Mol Cell Probes. 1993;7(2):139-44.
22. Kettner M, Navarova J, Langsadl L. Aminoglycoside resistance patterns in clinical isolates of *Enterobacteriaceae* from Czechoslovakia. J Antimicrob Chemother. 1987;20(3):383-7.
23. CLSI. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-First Informational Supplement. CLSI document M100-S21. Wayne, PA: Clinical and Laboratory Standards Institute; 2011.
24. Hanahan D. Studies on transformation of *Escherichia coli* with plasmids. J Mol Biol. 1983;166(4):557-80.
25. Nicolle LE, Committee ACG. Complicated urinary tract infection in adults. Can J Infect Dis Med Microbiol. 2005;16(6):349-60.

26. Turnidge J, Bell J, Biedenbach DJ, Jones RN. Pathogen occurrence and antimicrobial resistance trends among urinary tract infection isolates in the Asia-Western Pacific Region: report from the SENTRY Antimicrobial Surveillance Program, 1998-1999. Int J Antimicrob Agents. 2002;20(1):10-7.
27. Gad GF, Mohamed HA, Ashour HM. Aminoglycoside resistance rates, phenotypes, and mechanisms of Gram-negative bacteria from infected patients in upper Egypt. PLoS One. 2011;6(2):e17224.
28. Mathai D, Jones RN, Pfaller MA, America SPGN. Epidemiology and frequency of resistance among pathogens causing urinary tract infections in 1,510 hospitalized patients: a report from the SENTRY Antimicrobial Surveillance Program (North America). Diagn Microbiol Infect Dis. 2001;40(3):129-36.
29. Gales AC, Sader HS, Jones RN, Group SP. Urinary tract infection trends in Latin American hospitals: report from the SENTRY antimicrobial surveillance program (1997-2000). Diagn Microbiol Infect Dis. 2002;44(3):289-99.
30. Bouza E, San Juan R, Munoz P, Voss A, Kluytmans J, Co-operative Group of the European Study Group on Nosocomial I. A European perspective on nosocomial urinary tract infections II. Report on incidence, clinical characteristics and outcome (ESGNI-004 study). European Study Group on Nosocomial Infection. Clin Microbiol Infect. 2001;7(10):532-42.
31. Poole K. Aminoglycoside resistance in *Pseudomonas aeruginosa*. Antimicrob Agents Chemother. 2005;49(2):479-87.
32. Dubois V, Arpin C, Dupart V, Scavelli A, Coulange L, Andre C, *et al*. Beta-lactam and aminoglycoside resistance rates and mechanisms among *Pseudomonas aeruginosa* in French general practice (community and private healthcare centres). J Antimicrob Chemother. 2008;62(2):316-23.
33. Vaziri F, Peerayeh SN, Nejad QB, Farhadian A. The prevalence of aminoglycoside-modifying enzyme genes (*aac (6')-I*, *aac (6')-II*, *ant (2")-I*, *aph (3')-VI*) in *Pseudomonas aeruginosa*. Clinics (Sao Paulo). 2011;66(9):1519-22.
34. Over U, Gur D, Unal S, Miller GH, Aminoglycoside Resistance Study G. The changing nature of aminoglycoside resistance mechanisms and prevalence of newly recognized resistance mechanisms in Turkey. Clin Microbiol Infect. 2001;7(9):470-8.
35. Akalin HE, Torun M, Alacam R. Aminoglycoside resistance patterns in Turkey. Scand J Infect Dis. 1988;20(2):199-203.
36. Miller GH, Sabatelli FJ, Hare RS, Glupczynski Y, Mackey P, Shlaes D, *et al*. The most frequent aminoglycoside resistance mechanisms--changes with time and geographic area: a reflection of aminoglycoside usage patterns? Aminoglycoside Resistance Study Groups. Clin Infect Dis. 1997;24(1):46-62.
37. Shimizu K, Kumada T, Hsieh WC, Chung HY, Chong Y, Hare RS, *et al*. Comparison of aminoglycoside resistance patterns in Japan, Formosa, and Korea, Chile, and the United States. Antimicrob Agents Chemother. 1985;28(2):282-8.
38. Sabtcheva S, Kaku M, Saga T, Ishii Y, Kantardjiev T. High prevalence of the *aac(6')-Ib-cr* gene and its dissemination among *Enterobacteriaceae* isolates by CTX-M-15 plasmids in Bulgaria. Antimicrob Agents Chemother. 2009;53(1):335-6.
39. Kim SY, Park YJ, Yu JK, Kim YS, Han K. Prevalence and characteristics of *aac(6')-Ib-cr* in AmpC-producing *Enterobacter cloacae, Citrobacter freundii,* and *Serratia marcescens*: a multicenter study from Korea. Diagn Microbiol Infect Dis. 2009;63(3):314-8.

40. Stanisich VA. Identification and Analysis of Plasmids at the Genetic Level, in: Grinsted J, Bennett PM, editors. Methods in microbiology, Plasmid technology. 2nd ed. London: Academic Press Limited; 1988.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=246&id=8&aid=1956