Detection of Plasmid-Mediated 16S rRNA Methylase Conferring High-Level Resistance to Aminoglycosides in Gram negative bacilli from Egypt

Aisha Abu Aitta[1]; Manal El Said[1], Ehab El Dabaa[2]; Mohamed Abd El Salam[3] and Hesham Mohamed Mahdy[3]

[1]Microbiology Department, Theodor Bilharz Research Institute, Giza, Egypt.
[2]Biochemistry Department, Theodor Bilharz Research Institute, Giza, Egypt
[3]Medical Microbiology Department, Faculty of Science, Al-Azhar University, Cairo, Egypt
manalmicrobiology@yahoo.com

Abstract: Background: Recently, production of 16S rRNA methylases by Gram-negative bacilli has emerged as a novel mechanism for high-level resistance to aminoglycosides by these organisms in a variety of geographic locations. Therefore, the spread of high-level aminoglycoside resistance determinants has become a great concern. **Objective:** to determine the prevalence of the occurrence of 16S rRNA methylases genes in aminoglycoside-resistant gram-negative bacteria isolates. **Methods:** 30 Gram-negative bacilli amikacin resistant isolates were collected from inpatients and outpatients at Theodor Bilharz Research Institute (TBRI). Biochemical identification of bacterial species was performed with API 20E system. Antimicrobial susceptibilities of amikacin, gentamicin, tobramycin, neomycin, kanamycin, and netilmicin, were determined by the disk diffusion method and the MICs of amikacin were detected by the E test method. PCR was used to identify 16S rRNA methylas armA and rmtB genes. **Result:** *E.coli,* (15/30; 50%), *Klebsiella pneumonia* (7/30; 23.3%), *Pseudomonas aeruginosa* (4/30; 13.3%), *Enterobacter cloaca* and *Acinetobacter baumanii* (2/30; 6.7% each) were isolated. Extended spectrum β-lactamases (ESBLs) were detected in 7 (23.6%) isolates. Among the 30 isolates, 13 (43.3%) isolates showed a high level of resistance to amikacin. Seven out of 30 (23.3%) amikacin-resistant isolates were positive for 16S rRNA methylase genes. Six isolates (20%) were positive for *rmtB* gene and one (3.3%) *Enterobacter cloaca* ESBLs producer isolate was positive for *armA* gene. rmtB was detected in one (3.3%) *Pseudomonas aeruginosa*e isolate and five (16.7%) *E. coli* isolates, in which, 3 of them were ESBLs producers. ArmA and rmtB genes were not detected simultaneously. **Conclusions:** 16S rRNA methylase genes were detected in gram negative bacilli in TBRI. RmtB was found to be more prevalent than armA. There was correlation between the detection of methylase genes and the production of ESBLs.

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1. Introduction

Aminoglycosides are clinically effective agents for treating a broad range of life-threatening infections caused by Gram-negative pathogens, usually in combination with β-lactam agents (Yu *et al.,* 2010). These agents bind to the A site of the 16S rRNA of prokaryotic 30S ribosomal subunits and subsequently block bacterial growth through interference with protein synthesis (Wachino *et al.,* 2006).

Over the past few decades, there have been many studies conducted regarding the mechanisms of resistance to aminoglycosides. Amikacin was developed to suppress a variety of aminoglycoside-modifying enzymes from their accessing target sites, and therefore rare, amikacin-resistant bacteria could be expected (Kang *et al.,* 2008). The emergence of pan-aminoglycoside– resistant, 16S rRNA methylase–producing, gram negative bacteria has been increasingly reported in recent years (Yamane *et al.,* 2007).

Alteration of the 16S rRNA A site by these enzymes (designated ArmA, RmtA, RmtB, RmtC, RmtD, and NpmA) confers resistance to almost all aminoglycosides, by limiting the binding of these agents to ribosomal target sites following methylation of specific nucleotides. ArmA (Enterobacteriaceae, Acinetobacter spp.) and rmtB (Enterobacteriaceae) appear to be the most widespread and have been detected primarily in Asia and Europe (Fritche *et al.,* 2008).

During the last years, bacterial identification based on molecular methods, especially those including the sequencing of genes coding for ribosomal 16S rDNA, has become a very important tool in studying bacterial communities in samples (Head *et al.,* 1998). Nucleic acid-based detection systems including polymerase chain reaction (PCR) offer rapid and sensitive methods to detect the

presence of resistance genes and play a critical role in the elucidation of resistance mechanisms. During the last decade, they have expanded tremendously and became more accessible for clinical microbiology laboratories (Fluit *et al.,* 2001, and Ercolini, 2004).

Thus, the aim of this study was to determine the prevalence of the occurrence of 16S rRNA methylases in aminoglycoside-resistant gram-negative bacteria isolates in TBRI by using molecular detection of the 16S rRNA methylases genes.

2.Material and methods

Clinical isolates:

Between June 2010 and February 2011, 30 Gram negative amikacin resistant clinical isolates were collected from outpatient clinic, hospitalized and Intensive Care Unit (ICU) patients admitted to TBRI, Cairo, Egypt. Identification of bacterial isolates was done to the genus and species level by cultural characters and biochemical reactions using the API 20 E kit (Bio-Mérieux, France).

Antimicrobial susceptibility testing:

The antimicrobial susceptibilities of amikacin, kanamycin, gentamicin, neomycin, netilmicin and tobramycin were determined by the disk diffusion test using commercial disks (Mast Diagnostics, U.K.) according to the criteria recommended by the CLSI (CLSI, 2010) using E. coli ATCC 25922 as the control strain. MICs of amikacin were further determined by using E test (AB Bio Disk Solna, Sweden) method in accordance with the CLSI guidelines (CLSI, 2010). High-level resistance to amikacin was dentified as MICs >256 mg/L by E test (Fritche *et al.,* 2008).

β-Lactamase characterization:

ESBL production was detected by phenotypic tests, which require a screening step followed by confirmation as recommended by the CLSI guidelines (CLSI, 2010).

A- Screening test: The disk-diffusion method for antibiotic susceptibility testing was used to screen ESBL production. Each gram negative bacilli isolate was considered a potential ESBL-producer according to the CLSI recommendation, if the zone diameter of the following antibiotics result were as follows: cefpodoxime (30µg) ≤ 22 mm, ceftazidime (30µg) ≤ 22 mm, aztreonam (30µg) ≤ 27 mm and cefotaxime (30µg) ≤ 27 mm

B-Confirmatory test (Double disk synergy test): The following antibiotics were tested: cefoxitin (FOX; 30µg), cefotaxime (CTX; 30µg), ceftazidime (CAZ; 30µg), cefepime (FEP; 30µg), aztreonam (ATM; 30µg), amoxicillin-clavulanic acid (AMC; 30/10µg), piperacillin/tazobactam (TZP; 100/10µg) and ampicillin (AMP; 10µg) (Bio-Rad, France). Antibiotic disks were arranged in proximity (30mm centre to centre) with the AMC disk. Results of antimicrobial susceptibility were interpreted according to CLSI guidelines: FOX (S≥18), CTX (S≥23), CAZ (S≥18), FEP (S≥18), ATM (S≥22), AMC (S≥22), TZP (S≥21) and AMP (S≥17) (CLSI, 2010). Test organisms showing synergy between the AMC disk and any cephalosporin disk or the ATM disk and/or between TZP and FEP was diagnosed as ESBL-producing strains (Pitout *et al.,* 2003). The standard strain E. *coli* ATCC 25922 was used as negative control for the assay.

Detection of Methylase Genes:

The armA and rmtB genes were detected by PCR as described by Doil and Arkawa, 2007. Briefly, a fresh bacterial colony was suspended in 200 µL of sterile distilled water and heated to 95°C for 10 min in water bath. After centrifugation at 8500 rpm for 20 min; the supernatants were removed and stored at -20°C for PCR assay. Primers for armA gene amplification were 5̀ ATTCTGCCTATCCTAATTGG 3̀ and 5̀ ACCTATACTTTATCGTCGTC 3̀, which are specific for the flanking regions of the gene to produce a 315 bp product. Primers for rmtB gene amplification were 5̀ GCTTTCTGCGGGCGATGTAA 3̀ and 5̀ ATGCAATGCCGCGCTCGTAT 3̀ which are specific for the flanking regions of the gene to produce a 173 bp product. Reactions for both genes were run on a programmable thermal controller PTC-100^{tm} (MJ Research Inc., U. S. A) under the following conditions: initial denaturation at 95°C for 5 min, followed by 30 cycles at 95°C for 1min, at 55°C for 1min, at 72°C for 1 min, with a final extension at 72°C for 5 min and at 4°C for 10 min. PCR products were electrophoresed in 2.5% agarose gels and visualized under UV light.

Statistical Methods:

Data were statistically described in terms of frequencies (number of cases) and relative frequencies (percentages). A probability value (P value) less than 0.05 was considered statistically significant. All statistical calculations were done using computer programs Microsoft Excel 2007 (Microsoft Corporation, NY., USA), SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) version 15 for Microsoft Windows and Quick Calcs online calculators for scientists (Graph pad software Inc., San Diego, CA, USA)

3. Results

Specimens collected:

The study was included 30 clinical isolates of Gram negative bacilli that were collected from outpatient and inpatient of TBRI. They were 21 (70%) males and 9 females (30%). Their age was ranged from 10 to 80 years with mean age of 48.3 ± 17.1 years. The 30 amikacin-resistant Gram negative

bacilli isolates collected from outpatient and inpatient of TBRI were *E.coli,* (15/30; 50%), *Klebsiella pneumonia* (7/30; 23.3%), *Pseudomonas aeruginosa* (4/30; 13.3%), *Enterobacter cloaca* and *Acinetobacter baumanii* (2/30; 6.7% each) (Table 1).

Antimicrobial Susceptibility Testing:

Among the 30 amikacin-resistant clinical isolates of Gram negative bacilli, all *E.coli*, *Klebsiella pneumonia, Pseudomonas aeruginosa* and *Enterobacter cloaca* isolates were resistant to tobramycin, gentamicin, kanamycin and netilmicin. *Acinetobacter baumanii* isolates were resistant to tobramycin and kanamycin while they were equally sensitive to gentamicin and netilmicin (50%). *E.coli*, *Klebsiella pneumonia, Pseudomonas aeruginosa, Acinetobacter baumanii* and *Enterobacter cloaca* isolates were resistant to neomycin as follows: 40%, 85.7%, 75%, 50% and 100% respectively.

High-level resistance to amikacin (MICs >256 mg/L) among the 30 Gram negative isolates was detected in 43.4% (13/30) by E test. They were 7 *E. coli* isolates (23.4%), 3 *Klebsiella pneumoniae* isolates (10%), 2 *Pseudomonas aeruginosa* isolates (6.7%) and one *Enterobacter cloaca* isolates (3.3%). The MICs of amikacin for the remaining isolates ranged between 48 and 64 µg/ml.

β- Lactamase characterization:

Among the 30 amikacin resistant Gram negative bacilli isolates, 7 (23.3%) isolates were ESBL-producer and they were *E.coli* (3/7; 42.8%), *Klebsiella pneumoniae* (2/7; 28.6%), *Enterobacter cloaca* and *Acinetobacter baumanii* (1/7; 14.3%, each).

Prevalence of methylases genes:

Seven out of 30 (23.3%) amikacin-resistant isolates were positive for 16S rRNA methylase genes, among which 6 isolates (20%; 6/30) were positive for rmtB gene and only one isolate (3.3%;1/30) was positive for armA gene (figure 1). ArmA was detected in one *Enterobacter cloaca* isolate (50 %;1/2), and rmtB was detected in five (33.3 %; 5/15) *E. coli* isolates and one (25%; 1/4) *Pseudomonas aeruginosa*e isolate. ArmA and rmtB genes were not detected simultaneously.

ArmA gene was detected in one male patient (1/ 21; 4.8 %) while rmtB gene was detected in 5 (5/21; 23.8%) male patients and one (1/9; 11.1%) female patient. Their ages were ranged from 10 to 65 years. There was no statistically significant correlation between the detection of armA and rmtB genes and the sex of the patients (P value= 0.7 and P value=0.3, respectively). According to site of infection, armA-positive Gram negative bacilli isolates were detected in one urine (1/11; 9.1 %) sample, while rmtB positive isolates were detected in 2 urine (2/11; 18.2 %), 3 sputum (3/9; 33.3 %) and one wound (1/5; 20%) samples.

ArmA gene was detected in one ESBL-producer isolate (1/7; 14.3%), which was *Enterobacter cloaca*. RmtB gene was detected in 3 ESBL-producer isolates (3/7; 42.9%) and they were *E.coli* isolates. There was statistically significant correlation between the detection of methylase genes and the production of ESBLs (*P* value= 0.01).

All 16S rRNA methylase gene-positive isolates were highly resistant to amikacin (MICs >256 µg/mL). They were also resistant to gentamicin, tobramycin, kanamycin, netilmycin and neomycin (Table 2). The 16S rRNA methylase gene-positive isolates represent 53.8% (7/13) of the amikacin highly resistant Gram negative bacilli isolates. There was poor agreement between the phenotypic and genotypic characteristics (kappa= 0. 6).

All 16S rRNA methylase gene-positive isolates were susceptible to imipenem. ArmA gene positive *Enterobacter cloaca* isolate was resistant to amoxicillin/clavulanic, piperacillin/tazobactam, cefuroxime, cefoxitin, ceftazidime, cefoperazon, cefotaxime, cefepime, aztreoname, ciprofloxacin, levofloxacin, norfloxacin, ofloxacin, trimethoprim-sulfamethxazole and nitrofurantion.

The 5 *E.coli* rmtB positive isolates were resistant to amoxicillin and ceftazidime and also they were resistant to amoxicillin/clavulanic, cefotaxime, cefepime except one isolate. *Pseudomonas aeruginosa* rmtB positive isolate was resistant to amoxicillin, amoxicillin/clavulanic, cefuroxime, cefoxitin, cefoperazon, cefotaxime, aztreoname, nalidixic acid, ciprofloxacin, and levofloxacin, intermediate to ceftriaxone, and susceptible to piperacillin/tazobactam, ceftazidime, cefepime and meropenem.

4. Discussion

Aminoglycosides are broad-spectrum antibiotics of high potency that have been traditionally used for the treatment of serious Gram-negative infections. However, increasing resistance to aminoglycosides is becoming a serious clinical problem (Yamane *et al.,* 2005 and Gad *et al.,* 2011).

Resistance to aminoglycosides is frequently due to the acquisition of modifying enzymes such as acetyltransferases, phosphorylases and adenylyltransferases (Kotra *et al.,* 2000). Other mechanisms of resistance include ribosomal alteration and impaired uptake of the antibiotics (Yan *et al.,* 2004).

A series of special methylases that protect microbial 16S rRNA, the main target of aminoglycosides, was identified in several nosocomial pathogens (Yamane et al., 2007). Six plasmid-encoded 16S rRNA methylases, including

ArmA, RmtA, RmtB, RmtC, RmtD, and NpmA, have been identified in clinical isolates of Gram-negative bacilli from multiple geographic locations (Yu *et al.,* 2010).

Table (1): Distribution of the 30 amikacin-resistant Gram negative bacilli isolates According to Species, Specimen

Organism	Specimen					Department				
	Urine	Sputum	Wound Swab	Blood Culture	Ascitic Fluid	ICU	Urology	Surgery	Gastro enterology	Outpatient Clinic
E.coli.(no.15)	6	4	2	1	2	3	2	0	4	6
Klebsiella pneumoniae (no.7)	2	3	2	0	0	1	1	2	0	3
Pseudomonas aeruginosa (no.4)	1	1	1	1	0	2	1	0	0	1
Enterobacter cloaca (no.2)	1	1	0	0	0	1	0	0	0	1
Acinetobacter baumanii (no.2)	1	0	0	1	0	2	0	0	0	0
Total (no.30)	11	9	5	3	2	9	4	2	4	11

Type and Origin

Table (2): Phenotypic and Genotypic Characteristics of Positive Mythylases Isolates

Specimen	Organism	Gene	AN	GM	NET	TOB	K	N	E Test	ESBL
Urine	*Enterobacter cloaca*	ArmA	R	R	R	R	R	R	>256	P
Sputum	*E.coli*	RmtB	R	R	R	R	R	R	>256	P
Wound	*E.coli*	RmtB	R	R	R	R	R	R	>256	N
Urine	*E.coli*	RmtB	R	R	R	R	R	R	>256	P
Urine	*E.coli*	RmtB	R	R	R	R	R	R	>256	N
Sputum	*Pseudomonas aeruginosa*	RmtB	R	R	R	R	R	R	>256	N
Sputum	*E.coli*	RmtB	R	R	R	R	R	R	>256	P

AN (Amikacin), GM (Gentamicin), NET (Netilmicin), TOB (Tobramycin), K (Kanamycin), N (Neomycin), R (Resistant), P (Positive), N (Negative).

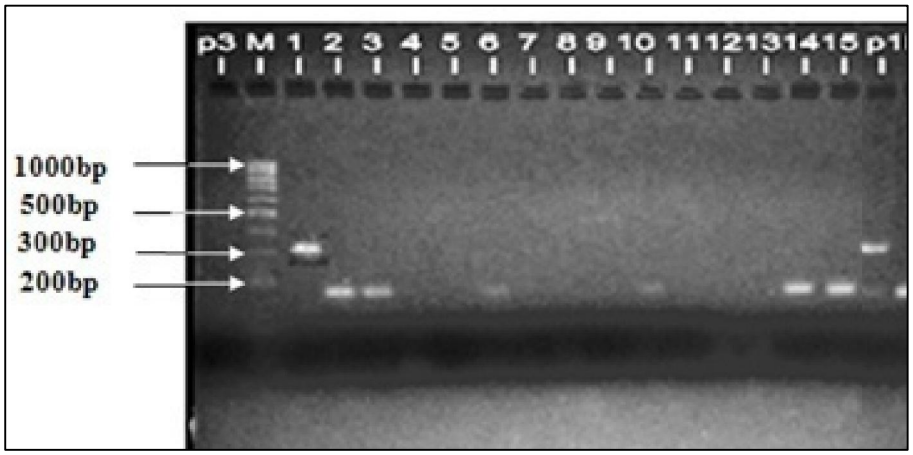

Figure (1): Detection of methylase genes in isolated gram negative bacilli: M=DNA weight (DNA ladder), P1=pooled positive 1, P3= pooled negative, and 1: 15 sample number

In this study, the prevalence of 16S rRNA methylases genes among the gram negative bacilli was determined by PCR. Thirty amikacin resistance Gram negative bacilli isolates were collected from outpatient and inpatient of TBRI. They were 21 (70%) males and 9 females (30%). The higher prevalence rate of aminoglycosides resistance in males (66.7%) than in females (33.3%) was also reported by Wassef *et al.*(2010) in an Egyptian study included 45 Gram negative bacilli that show resistance to all aminoglycosides in the period from March to June 2008 in Kasr El-Aini hospital, Cairo.

The majority of isolates were recovered from urine specimens (36.7%). The remaining isolates were recovered from sputum (30%), wound swab (16.7%), blood culture (10%) and ascitic fluid (6.6%). This rate was comparable with another Egyptian study at Minia University Hospital in Egypt by Gad *et*

al. (2011) who tested the aminoglycoside resistance in 175 Gram-negative bacteria isolates in the period from 2007 to 2009 and reported that the urinary isolates were 34.2 %, while wound swab isolates were 17.7%. Batchoun *et al.*(2009) reported that urine was the major source of the bacterial isolates collected, comprising 56% (262/472) of the total isolates and isolates from swabs and blood culture were 16.9% (80/472) and 16.7% (79/472) respectively.

E.coli was the most frequently isolated species (50%), followed by *Klebsiella pneumoniae* (23.3%), then *Pseudomonas aeruginosa* (13.3%). *Enterobacter cloaca* and *Acinetobacter baumanii* were equally isolated (6.7% each). Similar finding have been reported in the two Egyptian studies by Wassef *et al.* (2010) and Gad *et al.* (2011) who noted that, the most frequently isolated aminoglycosides resistance species was *E.coli* (39.6% and 28.57%) followed by *Klebsiella pneumoniae* (38.6%), then *Pseudomonas aeruginosa* (13.4% and 25.7%) respectively.

E.coli was isolated from urine, sputum, wound swab, blood culture and ascitic fluid specimens in percent of 40%, 26.7%, 13.3%, 6.7% and 13.3% respectively. Similar results were obtained by Wassef *et al.* (2010), who reported an isolation rate of 39.6% of *E.coli* from urine. Gad *et al.* (2011) noted slightly higher isolation rate of *E.coli* from urine (50%) while in skin infection was comparable with our results (12%).

Klebsiella pneumoniae was equally (28.6%) isolated from urine and wound swab specimens. *Pseudomonas aeruginosa* was equally isolated (25%) from urine, sputum, and wound swab and blood culture specimens. These results are in accordance with the findings of Gad *et al.* (2011) who reported equal isolation of *Klebsiella pneumoniae* from urine and wound swab specimens (37.5%) while the isolation rate of *Pseudomonas aeruginosa* from urine and skin infection were 22.2% and 20% respectively.

In our study, 7 (23.3%) isolates were ESBL-producer. A similar finding was reported by Batchoun *et al.* (2009) who noted that, out of the 472 gram-negative isolates included in their study, 108 (22.9%) isolates were ESBL producers. However, very high isolation rate 93.3% (42/45) was detected in Wassef *et al.* (2010) study. This may be attributed to that ESBLs was detected in the 45 gram-negative isolates that showed high-level amikacin resistance, while in our study it was detected in all 30 amikacin resistance gram-negative isolates regardless the level of resistance.

In the present study, ESBLs-producer isolates were *E.coli* (42.8%), *Klebsiella pneumoniae* (28.6%) and *Enterobacter cloaca* and *Acinetobacter baumanii* (14.3%, each). Many studies reported the detection of ESBLs-production isolates in *E.coli* (39.1%) (Moyo *et al.,* 2010), *Klebsiella pneumoniae* (17.2%) (Gangoué-piéboji *et al.,* 2005) and *Enterobacter cloacae* (13.9%) isolates (Batchoun *et al.,* 2009).

In this study, *E.coli*, *Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Enterobacter cloaca* were resistant to amikacin, gentamicin, tobramycin, kanamycin, and netilmycin antibiotic discs. Similar findings were reported by Gad *et al.* (2011). It was found that 46.6 % of *E. coli* isolates and 33.3% of *K. pneumoniae* isolates demonstrated high-level resistance to amikacin (MICs >256 mg/L) in our study. These results are comparable with the findings of Yan *et al*. (2004) who reported that, 50% of *E. coli* isolates and 39.6% *of K. pneumoniae* isolates demonstrated high-level resistance to amikacin.

On the other hand, 50% (1/2) of *E. cloacae* isolates demonstrated high-level resistance to amikacin (MICs >256 mg/L) in this study while Kang *et al.* (2008) reported lower prevalence (8.7%) in a study done in a university hospital in South Korea between 1995 and 2006. The high prevalence of highly-level resistance *E. cloacae* isolates in our study could be attributed to lower number of samples in comparison to the larger numbers of the isolates and the longer duration of the South Korean study. However, the prevalence in some periods of this study was elevated to 31% in 1997 and to 40% in 1998.

In this study *Pseudomonas aeruginosa* demonstrated high-level resistance to amikacin (50%). This result was higher than that described in a Japanese study (4.4%) presented by Yamane *et al.* (2007). Their low prevalence rate could be referred to higher geographic distribution of clinical specimens collected from 16 hospitals and the higher number of isolates (384) in comparison to lower numbers (2/4) in our study. Yamane *et al.,* 2007 used a different method including LB agar plates supplemented with 500 mg of arbekacin per liter while in our study amikacin E test was used.

In our study, no *Acinetobacter baumannii* isolates demonstrated high-level resistance to amikacin. Similar results were obtained from a study conducted on *Acinetobacter* isolates collected from Europe, North America, and Latin America. They reported no detection of methyltransferases among the *Acinetobacter* species isolates during the study period from 2005 to 2006 (Fritsche *et al.,* 2008). In contrast, a Japanese study presented by Yamane *et al.* (2007), reported a higher level of resistant (12.1%) among *Acinetobacter* species isolates. Also, Doi *et al.* (2007) reported a detection of five highly amikacin-resistant *Acinetobacter baumannii* isolates in a study done between December 2006 and March 2007 at a medical center in North America. They reported that

the aminoglycoside resistance was due to the production of the 16S rRNA methylase ArmA.

ArmA gene was detected in one *Enterobacter cloaca* isolate out of 30 amikacin resistance isolates (3.3%; 1/30). These results are similar to the findings o,-f Yamane et al. (2007) who reported that amA gene was detected in one *E. cloaca* isolate out of 29 highly resistance isolates to amikacin (3.4%; 1/29). Also, Kang *et al.* (2008) reported that the detection rate of armA in *Enterobacter cloacae* was 4.1% (19/463) in a study done to detect the 16SrRNA methylase genes in amikacin resistant Enterobacteriaceae isolates that were collected in 1995 to 2006 at university hospital in South Korea. Other studies reported a detection rate of armA in *Enterobacter cloaca* isolates of 2.9% (1/34) and 2.98% (6/201) respectively (Galimand *et al.,* 2005 and Wu *et al.,* 2009). However, Fritsche *et al.* (2008) reported higher prevalence of armA gene as it was detected in two *Enterobacter cloaca* isolates out of 22 amikacin resistance isolates (9%; 2/22).

In the present study, the prevalence of armA among *Enterobacter cloacae* was 50% (1/2) and was isolated from urinary sample. These results are in accordance with Kang *et al.* (2008) who reported that the detection rate of armA among *Enterobacter cloacae* isolates was 45.2% (19/42) in a long period study done in amikacin resistant Enterobacteriaceae isolates that were collected in 1995 to 2006 at university hospital in South Korea. However, Galimand *et al.* (2005) and Yamane *et al.* (2007) reported that a higher prevalence rate 100% (1/1) was detected among amikacin resistant *Enterobacter cloacae* isolates. This may be attributed to the small numbers of isolates used in these studies. Yamane *et al.* (2007) noted that the armA-positive *Enterobacter cloacae* isolate was detected in a urinary sample. Wu *et al.* (2009) reported that the prevalence rate of armA in *Enterobacter cloacae* was 25% (6/24) in Shanghai, China. This lower prevalence rate of armA may be due to the higher samples size in this study.

In the present study, the prevalence of rmtB gene in amikacin resistant isolates was 20%. These results are in accordance with Kang *et al.* (2008) who reports that the detection rate of rmtB was 23.3 % (51/218) among amikacin resistant isolates. Nearly similar to our results, Wassef *et al.* (2010) who reported that the detection rate of rmtB was 15.1% among 45 amikacin resistant isolates. Different prevalence rates of rmtB gene in amikacin resistant isolates were reported, in Taiwan it was 8.6% (Yan *et al.,* 2004), 36.4% in North and South America Fritsche *et al.,* 2008 and in China were 10.9% (Wu *et al.,* 2009) and 84% (Yu *et al.,* 2010).

In our study, rmtB was detected in five *E. coli* (5/15; 33.3%) amikacin-resistant isolates. These results are comparable with Kang *et al.* (2008) who reported that the RmtB was detected in 34.6% (18/52) of amikacin resistant *E. coli* isolates. However, *Yu et al.* (2010) reported higher rmtB gene detection rate (81.8%; 36/44) which indicates higher spread of rmtB gene carrying plasmid in the isolated *E. coli*. One (1/4; 25%) *Pseudomonas aeruginosa* isolate was rmtB positive in our study. Similar finding was reported by a Japanese study presented by Yokoyama *et al.* (2003). Other studies reported that no rmtB gene was detected among the amikacin resistant *Pseudomonas aeruginosa* isolates (Yamane *et al.,* 2007 and Fritsche *et al.,* 2008).

In our study, no rmtB gene was detected in *Klebsiella pneumoniae*, *Enterobacter cloaca* or *Acinetobacter baumanii* isolates. Similar findings were reported by many studies (Doi *et al.,* 2007, Yamane *et al.,* 2007 and Kang *et al.,* 2008). However, some studies reported that rmtB was detected in *Klebsiella pneumoniae* in rates of 8.6% and 20% respectively (Yan *et al.,* 2004 and Fritsche *et al.,* 2008).

In our study, rmtB gene-positive *E.coli* isolates were isolated from 2 sputum (40%); 2 urinary (40%) and (20%) one wound samples. Nearly similar finding were reported by Yu *et al*.(2010) who noted that rmtB gene-positive isolates was detected in 36 clinical samples in a rate of 44.4% and 27.8% for urinary and pus samples respectively, however, lower prevalence of rmtB in sputum samples (2.8%; 1/36) was detected.

In our study, the associations between 16S rRNA methylase and ESBLs production were detected in a rate of 66.7%. The armA-positive *Enterobacter cloacae* isolate was ESBLs producers and the three (60%; 3/5) rmtB- positive *E.coli* isolates were ESBLs producers. Many studies reported the associations between 16S rRNA methylase and ESBLs production with different rates ranging from 78.4% (Yan *et al.,* 2004), 94.3% (Yu *et al.,* 2010) to 100% (Wassef *et al.,* 2010)

In our study, all 5 *E. coli* isolates harboring 16S rRNA methylase genes were resistant to ciprofloxacin and susceptible to imipenem. Similar results were reported in Chinese study presented by Yu *et al.* (2010) who noted that all isolates harboring 16S rRNA methylase genes were resistant to ciprofloxacin, and susceptible to imipenem. Yan *et al.* (2004) reported that all armA-positive and rmtB positive isolates recovered were susceptible to imipenem. However, nine of the 28 (32.1%) armA-positive isolates and six of the seven (85.7%) rmtB positive isolates were resistant to ciprofloxacin. This could be explained by the use of MIC method to determine the susceptibility of isolates to ciprofloxacin in the Taiwanese study while in our

study disc diffusion method was used. This Clinical data supports the use of carbapenems for treatment of infections due to 16S rRNA methylase associated with ESBL-producing organisms. However, in a study in Pennsylvania presented by Doi *et al.* (2007), armA was detected in two *Acinetobacter baumannii* isolates coproduced OXA-23 β-lactamase and were highly resistant to carbapenems.

In our study, rmtB was more prevalent (20%; 6/30) than armA (3.33%; 1/30) among amikacin resistance isolates. Similar results were obtained from china by Doi *et al.* (2004) and Wu *et al.* (2009) who reported that rmtB was found to be more prevalent than armA in their hospitals. However, Yan *et al.*(2004) in a Taiwanese study and Wassef *et al.* (2010) in an Egyptian study suggest that armA is more prevalent than rmtB amongst Enterobacteriaceae isolates. Wassef *et al.* (2010) attributed the apparently higher prevalence of armA compared with that of rmtB in his study and the Taiwanese study to its association on the same conjugative plasmid with the gene for CTXM- 3 and its location on the functional transposon Tn1548. It worthy mentioned that ESBLs isolation rate was very high (93.3%) in Wassef *et al.* (2010) (16) study.

In our study there was poor correlation between the phenotypic pattern of aminoglycosides resistance detected by disk diffusion test and the genotypic pattern of resistance that was detected in (7/13; 53.8%) among high-level resistance to amikacin (MICs >256 mg/L) isolates. Similar finding was reported by Wassef *et al.* (2010) who noted a correlation in only 24.2% of bacterial isolate. On the other hand, a higher correlation record (95.5%) was detected in a surveillance study performed in Europe, North America, and Latin America. This could be attributed to the usage of nine aminoglycosides including arbekacin aminoglycoside modifying enzymes and a high-level arbekacin resistance (MIC >512 mg/L) was used as for screening the 16S rRNA methylase-producing strains (Pe´richon *et al.*, 2010). The findings reported by Wassef *et al.* (2010) was explained by the presence of other causes of aminoglycosides resistance in the PCR negative isolates such as the enzymatic modification and the absence of arbekacin in the selective criteria as arbekacin enhance the chance for detection of the 16S rRNA methyltransferases causative genes. This could be an acceptable explanation for our study also as the conditions were nearly similar.

Plasmid-mediated armA and rmtB genes have been identified from *E. coli* in swine from Spain and China (Gonzalez-Zorn *et al.*, 2005 and Chen *et al.*, 2007). In China, it was reported that the emergence of the armA and rmtB genes in clinical isolates of *E. coli* found in chickens (Du *et al.*, 2009). A large amount of aminoglycosides has been consumed in veterinary medicine. This may have served as a selective pressure for enteric gram-negative organisms to acquire 16S rRNA methylase genes, possibly from nonpathogenic environmental actinomycetes that intrinsically produced aminoglycosides or similar 16S rRNA inhibitors, and then maintain and spread them to humans through the food supply chains (Doi and Arakawa, 2007).

Conclusion

16S rRNA methylase genes were detected in gram negative bacilli in TBRI. RmtB was found to be more prevalent than armA. There was correlation between the detection of methylase genes and the production of ESBLs the aminoglycoside resistance produced by methylase and the association of their genes with mobile elements will require enhanced laboratory capabilities for their detection, appropriate infection control practices to limit continued spread, and reliance on alternative chemotherapeutic agents including carbapenems for treatment of infections due to 16S rRNA methylase associated with ESBL-producing organisms. The developments of new aminoglycoside agents that bind to methylated ribosomes are critically needed.

Corresponding authors
Manal El Said
Microbiology Department, Theodor Bilharz Research Institute, Giza, Egypt. Theodor Bilharz Research Institute
manalmicrobiology@yahoo.com

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