Infection and Drug Resistance

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ORIGINAL RESEARCH

High Rates of Aminoglycoside Methyltransferases Associated with Metallo-Beta-Lactamases in Multidrug-Resistant and Extensively Drug-Resistant *Pseudomonas aeruginosa* Clinical Isolates from a Tertiary Care Hospital in Egypt

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Background: Multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains of *Pseudomonas aeruginosa* are the leading cause of healthcare-associated infections worldwide.

Objective: The aim was to identify the resistant phenotypes among *P. aeruginosa* and to characterize different aminoglycosides and carbapenem resistance genes as major mechanisms of resistance in these isolates, in Theodor Bilharz Research Institute (TBRI), a tertiary care hospital in Cairo, Egypt.

Methods: During a period of 11 months, 42 *P. aeruginosa* clinical isolates were collected from the microbiology laboratory by routine culture. Antimicrobial sensitivity testing to the aminoglycosides gentamicin and amikacin, and other classes of antibiotics, was performed by a disk diffusion method. Isolates were tested for aminoglycoside resistance genes, *aac(6')-lb*, *aac-(3)-lla*, *rmtB*, *rmtC*, *armA*, *rmtD*, and *rmtF*, and carbapenemase resistance genes bla_{NDM}, bla_{VIM}, and bla_{IMP}, using conventional PCR.

Results: Thirty-three (78.5%) of the clinical *P. aeruginosa* isolates showed MDR and XDR phenotypes at 42.4% and 57.65%, respectively, and these were included in the study. Aminoglycoside resistance was found in 97%, whereas carbapenem resistance was found in 81% of the isolates phenotypically. Only 59.4% (19/26) of the aminoglycoside-resistant isolates harbored resistance genes; none of the amikacin-susceptible isolates harbored any of the tested aminoglycoside resistance genes. Aminoglycoside resistance genes *rmtB*, *armA*, *aac(6')-lb*, and *rmtF* were found at rates of 17/33 (51.5%), 3/33 (9%), 2/33 (6%), and 2/33 (6%), respectively, whereas *rmtD*, *acc(3)-II*, and *rmtC* were not detected. Only 40.7% (11/27) of the carbapenem-resistant isolates harbored resistance genes. Carbapenem resistance genes, bla_{NDM} andbla_{VIM}, were found at rates of 7/33 (21.2%) and 6/33 (18.1%), respectively, and bla_{IMP} was not detected.

Conclusion: Rates of MDR and XDR *P. aeruginosa* and resistance to aminoglycosides and carbapenems in our setting are high. Methyltransferases and metallo-beta-lactamases are the main mechanisms of resistance to aminoglycosides and carbapenems, respectively. The presence of bla_{NDM} and *rmtF* in the strains confirms their rapid dissemination in the Egyptian environment.

Keywords: aminoglycosides, carbapenems, antibiotic resistance, *Pseudomonas aeruginosa*, MDR, XDR, *rmtB*, *rmtF*, NDM

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Introduction

Pseudomonas aeruginosa is a Gram-negative, rod-shaped, strictly aerobic bacterium. This bacterium is an opportunistic pathogen; it causes serious acute and persistent infections that often occur during existing diseases or conditions, including cystic fibrosis and traumatic burns. It is considered a cornerstone pathogen among the important resistant ESKAPE bacteria (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *P. aeruginosa*, and *Enterobacter* species), which are the main pathogens for community and hospital drug resistance.[1] It is also placed at the top of the WHO's ranking list of critical pathogens for which there is a need to develop and discover novel therapeutic modalities.[2] *Pseudomonas aeruginosa* is the cause of about 10% of hospital-acquired infections worldwide, leading to outbreaks in adult, pediatric, and neonatal intensive care units (ICUs) due to the spread of multidrug-resistant (MDR) or extensively drug-resistant (XDR) strains. Thus, it increases morbidity, mortality, length of hospitalization, and treatment costs. Antibiotics against *P. aeruginosa* include beta-lactam/beta-lactamase inhibitors, such as piperacillin–tazobactam, third generation cephalosporin (ceftazidime), fourth-generation cephalosporin (cefepime), carbapenems (imipenem and meropenem), aminoglycosides (amikacin, gentamicin, and tobramycin), fluoroquinolones (ciprofloxacin, ofloxacin, and levofloxacin), monobactam (aztreonam), and colistin.[2]

Among the eight categories of anti-pseudomonal agents, aminoglycosides are widely used to treat acute and chronic infections.[3] During the past few years, increased bacterial resistance to aminoglycosides with anti-pseudomonal activities, including gentamicin, tobramycin, and amikacin, has been documented among healthcare-associated bacterial pathogens in almost all parts of the world.[4] The identification of aminoglycoside resistance mechanisms is thus needed to avoid therapeutic failures, and is a prerequisite for the development of novel and innovative inhibitory molecules.[5]

There are four main mechanisms for aminoglycoside resistance: aminoglycoside-modifying enzyme (AME) production, reduced cell permeability and uptake, and modification of the ribosomal binding site.[6] However, enzymatic modification of hydroxyl and amino groups is considered the most common mechanism.[7] Modifying enzyme genes result in reduced binding of the aminoglycoside molecule to the ribosome. There are three families of aminoglycoside-modifying enzymes, namely, aminoglycoside acetyltransferases (AACs), aminoglycoside phosphotransferases (APHs), and aminoglycoside nucleotidyltransferases (ANTs).[7] AACs constitute the main type of AME in *P. aeruginosa*.[8]

Carbapenems are among the primary treatment options for serious *P. aeruginosa* infection. The evolution of carbapenem-resistant *Pseudomonas aeruginosa* (CRPA) strains, mediated by acquiring genes encoding class B enzymes, is a global concern. CRPA occurs mainly through carbapenem-hydrolyzing enzymes. The metallo-beta-lactamases (MβLs) (eg, Verona integron-encoded metallo-beta-lactamases [VIMs], imipenemases [IMPs], and New Delhi metallo-beta-lactamases [NDMs]) are considered to be the most important enzymes in *P. aeruginosa*. The *bla* genes encoding these enzymes are either plasmid or chromosome mediated, usually located as horizontally transferable cassettes that classically cluster with other drug resistance determinants. So, the spread of *P. aeruginosa* with MβL activity could result in a pan-resistant phenotype, thus resulting in further limitation of therapeutic treatment options for these isolates.[9]

In this study, we aimed to evaluate the predominance of aminoglycoside resistance among MDR and XDR *P. aeruginosa* isolates, and to detect the main mechanisms of resistance and the most prevalent aminoglycoside resistance genes. Since carbapenem resistance represents a crucial problem that further limits therapeutic options in these isolates, $bla_{\text{M}\beta\text{L}}$s were also investigated.

Materials and Methods

Clinical Specimens and Culture Conditions

From January 2020 to November 2020, 42 non-duplicate *P. aeruginosa* isolates were collected from different clinical specimens (urine, sputum, pus, and blood) obtained from inpatients and outpatients attending the microbiology laboratory at Theodor Bilharz Research Institute (TBRI), a tertiary care hospital in Cairo, Egypt. All specimens included in the study were archived and codes were used instead of patients' names. The patients' informed consent was waived as all patient data were anonymized. The protocol of the study was approved by TBRI institutional review board under Federal Wide Assurance (FWA00010609) and the work was carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) for Experiments in Humans and its later amendments (GCP guidelines) or comparable ethical standards.

Identification of *P. aeruginosa* was carried out using standard techniques, and biochemical tests were conducted according to the procedures and protocols for the identification of non-fermenting Gram-negative bacilli. *Pseudomonas aeruginosa* identification was based on Gram staining, colony morphology, motility, pigment production, and oxidase reaction (HiMedia, India). Identification was confirmed using API 20 NE (Biomerieux, France). *Pseudomonas aeruginosa* ATCC 27853 was used as a positive control for bacterial identification. The *P. aeruginosa* isolates were stored in the form of glycerol stocks in sterile Eppendorf tubes and kept at −70°C until processed.[10]

Phenotypic Detection and Susceptibility Testing

Susceptibility testing for *P. aeruginosa* strains was carried out by the Kirby–Bauer disk diffusion method on Mueller–Hinton agar plates, against gentamicin (GM) (10 µg), amikacin (AK) (30 µg), ofloxacin (OFX) (5 µg), ciprofloxacin (CIP) (5 µg), levofloxacin (LEV) (5 µg), norfloxacin (NOR) (10 µg), ceftazidime (CAZ) (30 µg), cefepime (FEP) (30 µg), piperacillin/tazobactam (TZP) (100/10 µg), aztreonam (ATM) (30 µg), meropenem (MEM) (10 µg), and imipenem (IPM) (10 µg). The zone of inhibition was measured after 24-hour incubation at 35–37°C and interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines.[11] Resistance phenotypes were defined as MDR *P. aeruginosa* for an isolate that is non-susceptible to at least one agent in three or more antimicrobial categories; an XDR isolate is non-susceptible to at least one agent in all but two or fewer categories; and pan-drug resistant means that an isolate is non-susceptible to all antimicrobial agents.[12]

DNA Extraction from *P. aeruginosa* Isolates

Genomic DNA was extracted from pure colonies of isolated *P. aeruginosa* isolates using the boiling method. Bacterial colonies were suspended in 1 mL TE buffer (1 mM EDTA, 10 mM Tris, pH 8) and boiled for 10 minutes. Subsequently, the samples were kept at −70°C until processed.[13]

PCR Analysis of Aminoglycoside and Carbapenem Resistance Genes

Detection of *aac(6')-lb*, *aac-(3)-lla*, *rmtB*, *rmtC*, *armA*, *rmtD*, and*rmtF* genes, using a conventional PCR assay (Bio Rad T100 thermal cycler, USA), was performed in a volume of 20 µL, with approximately 2 µL of extracted DNA, using 1 µL (1 pmol/1 µL) of each primer (Invitrogen Co, USA), both forward and reverse primers (Table 1), nuclease-free water, and ready-made PCR master mix solution (Thermoscientific, USA). An initial denaturation at 95°C for 5 minutes was followed by 35 cycles of denaturation at 95ºC for 30 seconds, and then annealing at 59°C for *aac(6')-lb* gene, 61°C for *aac-(3)-lla* gene, 59°C for *rmtB* gene, 55°C for *rmtC* gene, 53°C for *armA* gene, 58°C for *rmtD* gene, 60°C for *rmtF* gene, 60°C for bla_{NDM} gene, 52°C for bla_{VIM} gene, and 52°C for bla_{IMP} gene, for 60 seconds. This was followed by extension at 72°C for 50 seconds. Final extension at 72°C for 10 minutes was the last step. The PCR for carbapenemase production genes, bla_{NDM}, bla_{VIM}, *and* bla_{IMP}, was performed as described before.[14] Each PCR product was separated on 2% agarose gel with ethidium bromide, and a 100-bp ladder was used as a DNA molecular weight standard. The sizes of the expected amplicons and sequences of primers are shown in Table 1.

Statistical Analysis

Statistical analysis was carried out using SPSS software (version 21.0) for Windows (#x1D712;^{2}-test). A p value of ≥0.05 was considered significant.

Results

Bacterial Isolates and Susceptibility Testing

Forty-two clinical *P. aeruginosa* isolates were collected from the microbiology laboratory at TBRI; 33 of these isolates showed resistance phenotypes, as follows: 14 (42.4%) were MDR, 19 (57.6%) were XDR, and they were all included in the current study. The resistance profiles of studied *P. aeruginosa* isolates showed that 97% (32/33) were aminoglycoside resistant, whether resistant to both gentamicin and amikacin, 78.8% (26/33), or resistant to gentamicin only, 18.1% (6/33), by the disk diffusion method. Isolates were recovered from different wards, including urology (10/33, 30.3%), outpatients (10/33, 30.3%), ICU (7/33, 21.2%), tropical (4/32, 12.1%), and nephrology (2/33, 6%). They were collected from different clinical specimens, including urine (22/33, 66.7%), sputum (4/33, 12.1%), pus (5/33, 15.1%), and blood (2/33, 6%).

The resistance of *P. aeruginosa* isolates against other tested antibiotics was as follows: OFX (31/33, 94%), CIP (31/33, 94%), LEV (30/33, 91%), CAZ (23/33, 69.7%), FEP (29/33, 88%), and TZP (21/32, 63.6%), whereas only

Table 1 Primers Used for the Detection of *aac(6')-Ib*, *aac(3)-IIa*, *rmtB*, *rmtC*, *armA*, *rmtD*, bla_{NDM}, bla_{VIM}, and bla_{IMP} Genes, and Expected Product Size

Gene Name	Sequence (5′–3′)	Amplicon Size	Reference
aac(6')-Ib	F: TTGCGATGCTCTATGAGTGGCTA R: CTCGAATGCCTGGCGTGTTT	482 bp	[15]
aac(3)-IIa	F: GGCAATAACGGAGGCGCTTCAAAA R: TTCCAGGCATCGGCATCTCATACG	563 bp	[15]
rmtB	F- GCTTTCTGCGGGCGATGTAA R- ATGCAATGCCGCGCTCGTAT	173 bp	[16]
rmtC	F- GCTGCCCTTTGTATTGTC R-AGATGTTGGGTTAAGTCCC	711 bp	[16]
armA	F- ATTCTGCCTATCCTAATTGG R- ACCTATACTTTATCGTCGTC	315 bp	[16]
rmtD	F- CGGCACGCGATTGGGAAGC R- CGGAAACGATGCGACGAT	401 bp	[16]
rmtF	F- GCGATACAGAAAACCGAAGG R-ACCAGTCGGCATAGTGCTTT	589 bp	[14]
bla_{NDM}	F-CCATGCGGGCCGTATGAGTGATTG R-TCGCGAAGCTGAGCACCGCATTAG	700 bp	[14]
bla_{VIM}	F- GATGGTGTTTGGTCGCATA R- CGAATGCGCAGCACCAG	390 bp	[14]
bla_{IMP}	F- GGAATAGAGTGGCTTAAYTCTC R- GGTTTAAYAAAACAACCACC	232 bp	[14]

7/33 (21.2%) were resistant to ATM. Carbapenem resistance was found in 81.8% (27/33) of the MDR *P. aeruginosa* isolates that were resistant to both imipenem and meropenem.

Molecular Detection of Aminoglycoside and Carbapenem Resistance Genes

Only 59.4% (19/26) of the aminoglycoside-resistant isolates harbored resistance genes; none of the amikacin-susceptible isolates harbored the tested aminoglycoside resistance genes.

Aminoglycoside resistance genes *rmtB*, *armA*, *aac(6')-Ib*, and *rmtF* were found at the following rates: 17/33 (51.5%), 3/33 (9%), 2/33 (6%), and 2/33 (6%), respectively, whereas *rmtD*, *acc(3)-II*, and *rmtC* were not detected (Table 2). The gene products of *rmtB*, *armA*, and *aac(3)-Ib* genes are shown in Figures 1–3.

Genes coding for methyltransferases (MTs) were the main resistance genes in aminoglycoside-resistant *P. aeruginosa* isolates. *rmtB* was the main gene and it was found alone in 14 isolates; they were collected from the ICU (4/14, 28.6%), outpatients (4/14, 28.6%), urology (3/14, 21.4%), nephrology (2/14, 14.2%), and tropical medicine departments (1/14, 7.1%); they were isolated from urine (11/14, 78.5%) and sputum (3/14, 21.4%). One isolate harbored *rmtB* + *armA* and another harbored *rmtF* + *armA*; both were collected from outpatient urine samples, whereas one isolate harbored only *rmtF*, from a pus specimen from the urology department. Aminoglycoside-modifying genes were not found alone in any of aminoglycoside-resistant isolates; one isolate harbored *rmtB* + *aac(6')Ib* and was collected from the ICU department from a blood sample. One isolate harbored *rmtB* + *armA* + *aac(6')Ib* and was collected from an outpatient urine sample. Only 40.7% (11/27) of carbapenem-resistant isolates harbored resistance genes. Carbapenem resistance genes, bla_{NDM} and bla_{VIM}, were found at rates of 7/33 (21.2%) and 6/33 (18.1%), respectively, while bla_{IMP} was not detected (Figures 4 and 5). Only one carbapenem-susceptible isolate harbored bla_{VIM}.

Table 2 Association Between Aminoglycoside Resistance and Carbapenem Resistance Genotypes in 19 Positive Aminoglycoside Resistance Genes in *P. aeruginosa* Clinical Isolates

Aminoglycoside Resistance Genotypes (n= 19)[a]	Carbapenem Resistance Genotypes (n=8)[b]	Type of Sample				Source of Specimens
		Urine (n=14)	Blood (n=1)	Pus (n=1)	Sputum (n=3)	
rmtB 14 (73.6%)	- bla_{NDM} + bla_{VIM}, 1 (12.5%) - bla_{VIM}, 2 (25%) - bla_{NDM}, 1 (12.5%)	11 (78.5%)	0 (0.0%)	0 (0.0%)	3 (21.4%)	ICU, 4 (28.6%) Urology, 3 (21.4%) Nephrology, 1 (7.1) Tropical, 2 (14.2%) Outpatient, 4 (28.6%)
rmtB + *armA* 1 (7%)	———	1 (100%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	Outpatient, 1 (100%)
rmtB + *aac(6')Ib* 1 (7%)	bla_{NDM}, 1 (12.5%)	0 (0.0%)	1 (100%)	0 (0.0%)	0 (0.0%)	ICU, 1 (100%)
rmtB + *armA* + *aac(6')-Ib* 1 (7%)	—	1 (100%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	Outpatient, 1 (100%)
rmtF+ *armA* 1 (7%)	bla_{NDM}, 1 (12.5%)	1 (100%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	Outpatient, 1 (100%)
rmtF 1 (7%)	bla_{NDM}, 1 (12.5%)	0 (0.0%)	0 (0.0%)	1 (100%)	0 (0.0%)	Urology, 1 (100%)

Notes: All parameters are represented as *F* (%) [frequency and percent]; data were analyzed by the χ^2 test. [a]Indicates the presence of aminoglycoside resistance genes, including the detected methyltransferases (*rmtB*, *armA*, and *rmtF*), either alone or in combination with the only detected aminoglycoside-modifying enzyme, *aac(6')Ib*. [b]The detected metallo-beta-lactamases responsible for carbapenem resistance, including bla_{NDM} and bla_{VIM}, either alone or combined.
Abbreviation: ICU, intensive care unit.

Eight *P. aeruginosa* isolates harbored genes for both aminoglycoside and carbapenem resistance (Table 2).

Discussion

Infection with MDR *P. aeruginosa* presents a major health challenge as it is a common cause of healthcare-associated infections that are usually life-threatening, such as ventilator-associated pneumonia, bacteremia, and urinary tract infections, as well as wound and soft-tissue infections, which lead to significant morbidity and mortality.[2]

Treatment of *P. aeruginosa* is hampered by its ability to develop resistance to multiple classes of antibacterial agents, even during the course of treatment.[17] *Pseudomonas aeruginosa* normally has low intrinsic antibiotic susceptibility, mediated through different mechanisms of resistance, such as inducible AmpC cephalosporinase, MexAB-OprM efflux pumps, and inducible MexXY efflux pump, together with its low outer membrane permeability and OXA-type oxacillinase. This is in addition to its tendency to acquire resistance genes and its inherently low cell membrane permeability.[2,18]

Aminoglycosides are among the most frequently prescribed antimicrobial agents against Gram-negative bacterial infections, including *P. aeruginosa*. The emergence of resistance to aminoglycosides in pathogenic Gram-negative bacteria is a growing concern. The aim of this

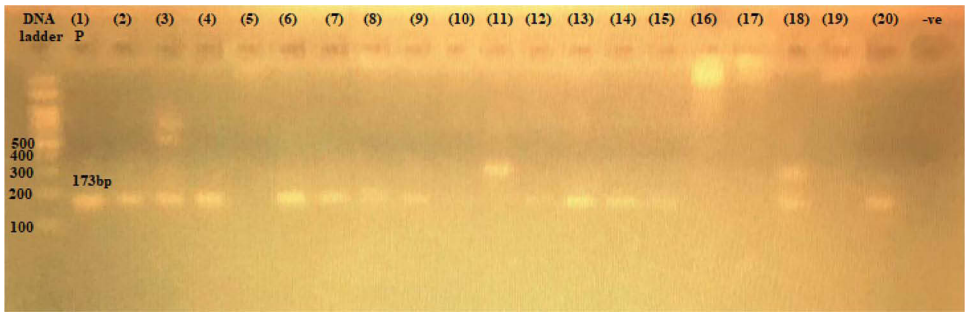

Figure 1 The 173-bp PCR amplification of *rmtB* gene among *P. aeruginosa* isolates. First lane, DNA ladder, 100-bp DNA size marker; (1) P, positive control, from 2 to 20 clinical isolates; −ve, negative control.

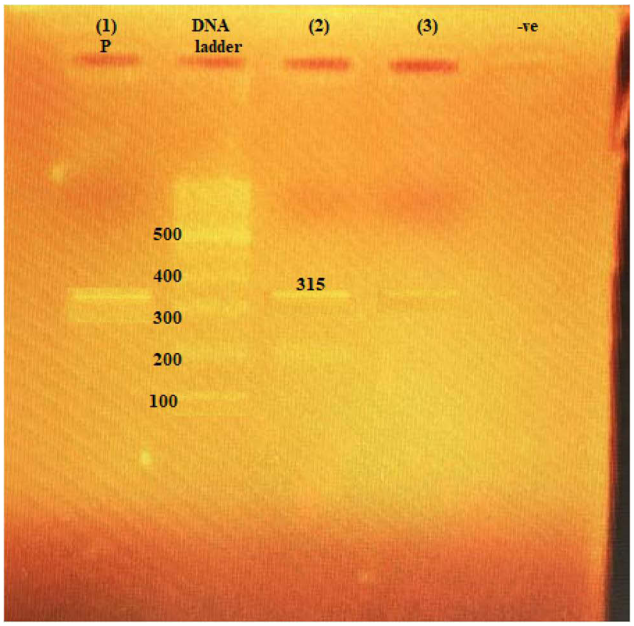

Figure 2 The 315-bp PCR amplification of *armA* gene among *P. aeruginosa* isolates. (1) P, positive control; second lane, DNA ladder, 100-bp DNA size marker; (2) and (3), clinical isolates with positive results; −ve, negative control.

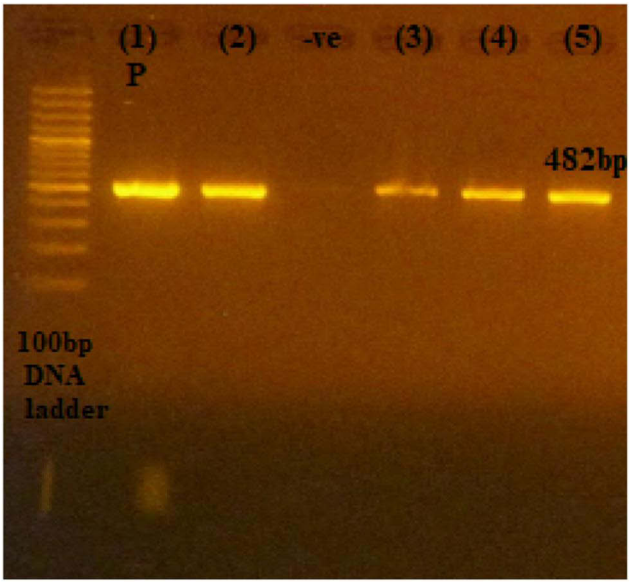

Figure 3 The 482-bp PCR amplification of *aac(6')-Ib* gene among *P. aeruginosa* isolates. First lane, DNA ladder, 100-bp DNA size marker; (1) P, positive control; (2), (3), (4), and (5), clinical isolates; −ve, negative control.

study was to identify the resistance phenotypes in *P. aeruginosa* and the prevalence of genes encoding resistance to aminoglycosides, as well as carbapenem resistance genes associated in the isolates, as a major mechanism of resistance in these isolates, from TBRI, a tertiary care hospital in Cairo, Egypt.

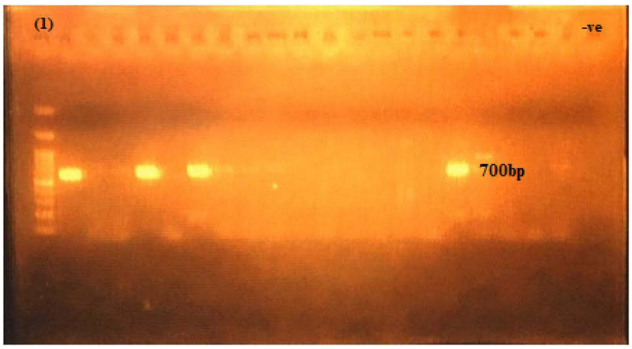

Figure 4 The 700-bp PCR amplification of bla_{NDM} gene among *P. aeruginosa* isolates. First lane, DNA ladder, 100-bp DNA size marker; −ve, negative control.

Figure 5 The 390-bp PCR amplification of bla_{VIM} gene among *P. aeruginosa* isolates. First lane, DNA ladder, 100-bp DNA size marker; −ve, negative control.

Thirty-three (78.6%) of MDR and XDR *P. aeruginosa* isolates were collected from different clinical samples received at the microbiology laboratory at TBRI during a period of 11 months (January to November 2020). This rate was comparable to that found in previous studies, where all *P. aeruginosa* isolates were found to be MDR in one study,[19] and 72.5% were MDR in Mansoura governorate in Egypt,[20] but much higher than the rates reported previously from Menofia governorate (19%)[21] and Upper Egypt (22.5%).[22] Research from Iran estimated the rates of MDR and XDR *P. aeruginosa* as 16.5% and 15.53%, respectively.[23] Another study showed that 88.9% of the isolates from Greece were MDR and XDR, whereas fewer isolates from Spain (33.3%) and Italy (43.5%) showed antibiotic resistance.[24] Isolates showed the highest resistance rates to fluoroquinolones (91–94%), while 69.7% were resistant to ceftazidime, 88% to cefepime,

63.3% to tazobactam–piperacillin, 21% to aztreonam, and 81.8% to carbapenems. Similar results were reported from neighboring countries, such as Bahrain, where resistance of 72–100% was found to third-generation cephalosporins, carbapenems, aminoglycosides, fluoroquinolones, and piperacillin–tazobactam. In a previous Egyptian study, aztreonam was the most effective antibiotic.[9] Another study from Europe showed that 64% of isolates were resistant to carbapenems and 54.7% of isolates were resistant to fluoroquinolones.[24] It is suggested that the rise in resistance to anti-pseudomonal antibiotics, especially carbapenems, aminoglycosides, and fluoroquinolones has contributed to the emergence of *P. aeruginosa* MDR/XDR strains, posing a serious and critical therapeutic situation.[20]

Urine was the main type of specimen from which isolates were collected, representing almost 67%, as in previous studies from Egypt and Iraq (both 100%), and from Saudi Arabia (88.9%), thus showing the difficulty in managing urinary tract infections secondary to MDR *P. aeruginosa*. Different results were found in other areas, such as in Lebanon, with a lower rate of MDR *P. aeruginosa* (30%).[25]

Resistance to aminoglycosides was found in 97% of our isolates; it was manifested either as resistance to both gentamicin and amikacin, in 79%, or as resistance to gentamicin only, in 18%. However, aminoglycoside resistance genes were detected only in 59% of amikacin and gentamicin-resistant isolates, indicating the possibility of the presence of another mechanism that confers resistance to different types of aminoglycosides, such as activated efflux pumps. This figure is comparable to another study from Egypt, where resistance to gentamicin and amikacin was 70% and 66%, respectively.[9] None of the susceptible isolates harbored aminoglycosides resistance genes, as in previous studies. A study from Saudi Arabia mentioned that the resistance profiles of *P. aeruginosa* isolates showed that 46.1% were resistant to one or more aminoglycoside antibiotics and that only 43.3% of the aminoglycoside-resistant isolates harbored resistance genes; none of the susceptible isolates harbored the tested resistance genes.[16]

Enzymatic modification of the aminoglycoside molecular structure with APH, AAC, or ANT AMEs represents the predominant mechanism of resistance in *P. aeruginosa* worldwide.[26] However, methyltransferases were the main aminoglycoside resistance genes identified in this study, with a predominance of *rmtB* (51.5%), followed by *armA* (9%) and then *rmtF* (6%). However, *aac(6')-Ib*, although known to be the commonest resistance gene among *P. aeruginosa* isolates, was detected only in two isolates (6%).[8] That rate of *rmtB* was much higher than those reported from Brazil (44.4%)[27] and from Saudi Arabia, where 43% of MDR *P. aeruginosa* isolates were resistant to amikacin and 18.5% to gentamicin, with *rmtB* also being the main resistance gene (7.6%), followed by *aac(6')-Ib* (6.1%), *rmtC* (4.6%), and *armA* (1.5%).[16] Our results confirm findings from the USA, showing that AMEs play a relatively minor role in aminoglycoside resistance.[28]

In the current study, a single aminoglycoside resistance gene was detected in 15 (45%) of the isolates, while a combination of two genes was found in three (9%), and the combination of three genes was detected in only one isolate (3%) of the 33 studied MDR *P. aeruginosa* strains (Table 2).

Among all studied methyltransferases tested in this study, *rmtF* represents the most recently discovered and the least reported globally[29,30]. Thus, *rmtF* has rarely been reported in *P. aeruginosa*, and, to our knowledge, this is the first time that it has been reported in Egypt in *P. aeruginosa* isolates. It has only been previously associated with NDM-1 production in Enterobacteriaceae isolates in Egypt.[14,30] In the current study, it was also found in two isolates that co-produced NDM enzyme. Aminoglycoside resistance is most frequently driven by aminoglycoside-modifying enzymes in *P. aeruginosa*, including the AACs and ANT. However, the prevalence of 16S rRNA MTs, which confer resistance to all aminoglycosides on the market, varies geographically, with the highest rates in Asia.[3,31] The predominance of MTs as the main aminoglycoside resistance genes in the Egyptian isolates is an essential finding, as it is known that these enzymes confer resistance to all aminoglycosides, including the novel ones such as plazomicin.[32]

The high resistance rate to carbapenems (81.8%), associated with relatively low resistance to aztreonam (21%), indicated that the production of MβLs is the main mechanism of resistance to carbapenems. Carbapenemase production genes, mainly bla_{NDM} (21%), followed by bla_{VIM} (18%), were detected. Our findings are comparable to a previous report from Egypt, where bla_{NDM-1} was the main MβL (90.9%), followed by bla_{VIM-1} (18.1%),[9] whereas bla_{IMP} was not detected, as in another previous

study which reported its low rate,[33] while other studies did not find it at all.[9,20,34] This is in contrast to other parts of the world, such as Iran and China, where bla_{IMP} is the prevailing MβL in *P. aeruginosa*.[35,36] The occurrence of bla_{VIM} in a carbapenem-sensitive isolate was also previously documented from our healthcare setting by another study.[37] However, carbapenem resistance MβL genes were found in only 40.7% of these isolates, with bla_{NDM} as a predominant carbapenemase-producing gene (21%), suggesting the possible presence of other mechanisms of resistance to carbapenems other than the production of carbapenemases. Carbapenem-resistant isolates harbored aminoglycoside resistance genes together with their carbapenem-resistant genes in seven isolates. The co-production of MTs and IMP- or VIM-type MβL is not common globally; however, such an association has been reported from Korea, Greece, and Sweden,[31] and from India.[30] In the absence of MβL enzymes, carbapenem resistance may be attributed to increasing production of AmpC chromosome-encoded cephalosporinase, increasing the expression of efflux pump permeability and loss of porins.[20]

Middle Eastern countries are recognized as crossroads, with a high volume of travel and varied nationalities and populations. They are also recognized for their high antibiotic consumption, despite endeavors to implement antimicrobial stewardship programs.[2] In the past few years, reports from Egypt have called again for the judicious use of conventional antimicrobials in an attempt to retain antibiotic effectiveness and avoid the spread of resistance genes.[19]

Conclusion

Our study confirms the prevalence of MTs as the main mechanisms of resistance to aminoglycosides in *P. aeruginosa* isolates, a finding that suggests the uselessness of all aminoglycosides in these isolates. This is in addition to the emergence of bla_{NDM} in *P. aeruginosa*, which is worrying as it further limits the choice of suitable antibiotics. These findings raise concern over the dynamic state of antimicrobial resistance genes and confirm our urgent demand for the rational use of antibiotics through effective antimicrobial stewardship programs and the need to follow strict infection control measures to avoid the spread of these resistant determinants. This was a single-institution study; in the future we intend to enlarge the sample size by collaboration with other health settings in Egypt, to determine the current situation at the country level.

Abbreviations

ESKAPE bacteria, *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species; ICU, intensive care unit; MDR, multidrug-resistant; XDR, extensively drug-resistant; AME, aminoglycoside-modifying enzyme; AAC, aminoglycoside acetyltransferase; APH, aminoglycoside phosphotransferase; ANT, aminoglycoside nucleotidyl transferase; CRPA, carbapenem-resistant *Pseudomonas aeruginosa*; MβL, metallo-beta-lactamase; VIM, Verona integron-encoded metallo-beta-lactamase; IMP, imipenemase; NDM, New Delhi metallo-beta-lactamase; TBRI, Theodor Bilharz Research Institute; CLSI, Clinical and Laboratory Standards Institute; PCR, polymerase chain reaction; MT, methyltransferase.

Data Sharing Statement

Data and material are available from the corresponding author upon reasonable request.

Ethical Considerations

All specimens included in the study were archived and codes were used instead of patients' names. The patients' informed consent was waived. The protocol of the study was approved by TBRI institutional review board under Federal Wide Assurance (FWA00010609) and the work has been carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) for Experiments in Humans and its later amendments (GCP guidelines) or comparable ethical standards.

Author Contributions

All authors made substantial contributions to conception and design, acquisition of data, analysis and interpretation of data; took part in drafting the article and revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work.

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Disclosure

The authors report no conflicts of interest for this work.

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