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Emergence of carbapenem resistant gram-negative pathogens with high rate of colistin resistance in Egypt: A cross sectional study to assess resistance trends during the COVID-19 pandemic

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ABSTRACT

The current study investigated the temporal phenotypic and genotypic antimicrobial resistance (AMR) trends among multi-drug resistant and carbapenem-resistant *Klebsiella pneumoniae*, *Acinetobacter baumannii,* and *Pseudomonas aeruginosa* recovered from Egyptian clinical settings between 2020 and 2021. Bacterial identification and antimicrobial sensitivity of 111 clinical isolates against a panel of antibiotics were performed. Molecular screening for antibiotic resistance determinants along with integrons and associated gene cassettes was implemented. An alarming rate (98.2%) of these isolates were found to be phenotypically resistant to carbapenem. Although 23.9 % *K. pneumoniae* isolates were phenotypically resistant to colistin, no mobile colistin resistance (*mcr*) genes were detected. Among carbapenem-resistant isolates, bla_{NDM} and bla_{OXA-48}-like were the most prevalent genetic determinants and were significantly overrepresented among *K. pneumoniae*. Furthermore, 84.78% of *K. pneumoniae* isolates co-produced these two carbapenemase genes. The plasmid-mediated quinolone resistance genes (*qnrS* and *qnrB*) were detected among the bacterial species and were significantly more prevalent among *K. pneumoniae*. Moreover, Class 1 integron was detected in 82% of the bacterial isolates. This study alarmingly reveals elevated resistance to last-resort antibiotics such as carbapenems as well as colistin which impose a considerable burden in the health care settings in Egypt. Our future work will implement high throughput sequencing-based antimicrobial resistance surveillance analysis for characterization of novel AMR determinants. This information could be applied as a step forward to establish a robust antibiotic stewardship program in Egyptian clinical settings, thereby addressing the rising challenges of AMR.

1. Introduction

Antimicrobial resistance is an increasing threat inflicting a challenge worldwide, which is intensified with the diminished development of new antimicrobials.[1] Recently, a growing concern has been raised raised for a potential increase in AMR secondary to misuse and overuse of antibiotics during the COVID-19 pandemic.[2] Multi-drug resistant (MDR) Gram-negative organisms, particularly carbapenem-resistant (CR) *Enterobacterales*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii* have impacted the rate of morbidity and mortality worldwide.[3–5] With the inefficacy of almost all the available antibiotics, there was an urge to find a solution for combating AMR.[5] Colistin, one of the polymyxin antibiotics group, was reintroduced as one of the remaining treatment options for life-threatening infections

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caused by multidrug and extensively drug-resistant Gram-negative bacteria. However, the increased use of colistin has led to a marked rise in the incidence of colistin-resistant infections.[6–7]

AMR mechanisms among different bacterial pathogens are broad,[8–9] whereas AMR traits can be acquired through horizontal gene transfer (HGT). AMR genes can be acquired from the gene pool by mobile genetic elements (MGEs) that can move between and within DNA molecules, such as gene cassettes, integrons, insertion sequences, and transposons.[9]

Integron allows transmission of resistance gene cassettes by site-specific recombination. They are usually located within MGEs such as plasmids and transposons, enhancing their dissemination and expression of AMR across wide range of bacterial pathogens.[10–11] Gene cassettes are free circular DNA sequences that are usually found on integrons. They are non-replicative, and contain an open reading frame, without a promoter but a recombination site (*attC*). Within the integron sequence, the integrase enzyme encoded by the *intI* gene recognizes the *attC* site of the gene cassette and catalyzes the insertion of the cassette into the integron at the recombination site known as *attI*. Integrons are classified into 3 classes; class 1, class 2, and class 3 which are associated with *intI1*, *intI2*, and *intI3*, respectively. Certainly, the interaction of these MGEs forms a complex system with the capacity of assembling and spreading resistance genes in bacterial populations.[9,12–13]

A recent study revealed that in low-middle-income countries (LMIC) especially the eastern Mediterranean region, CR proportions among *K. pneumoniae, A. baumannii,* and *P. aeruginosa* are considered higher than that of other LMIC, being the mostly isolated pathogens from hospital acquired infections in these regions.[14]

Thus, this study investigated the genotypic and phenotypic features of a total of 111 MDR *K. pneumoniae*, *A. baumannii,* and *P. aeruginosa* isolated from Egyptian clinical settings between 2020 and 2021 to outline the temporal AMR trends of these pathogens secondary to the intensive use of antibiotics and biocides at the onset of COVID-19 pandemic.

2. Methods

2.1. Bacterial isolates

One hundred and eleven non-duplicate Gram-negative isolates (including 46 *K. pneumoniae,* 45 *A. baumannii*, and 20 *P. aeruginosa*) were recovered from different clinical specimens (blood, urine, sputum, and respiratory secretions). Samples were obtained between August 2020 to April 2021 Mabaret El Asafra microbiology Lab that serves as a diagnostic center for many hospitals in Alexandria, Egypt. All samples were promptly transported to the laboratory in sterile containers within 12 h of collection. Isolation and enrichment steps were performed as previously described.[15] Species identification was confirmed using VITEK 2 Compact GN ID card (bioMérieux, Marcy-l'Étoile, France).

2.2. Antibiotic susceptibility testing

Antimicrobial susceptibility testing was performed using the Kirby-Bauer disk diffusion method using antibiotic disks (HiMedia laboratories Pvt Ltd., Mumbai) following the Clinical Laboratory Standards Institute (CLSI) guidelines.[16] Plates were incubated at 35°C and read after 16–20 h incubation, Inhibition zones were interpreted for 24 clinically used antibiotics (Table 1). Tested strains were defined as MDR if they exhibited resistance to at least one agent in three or more antimicrobial classes.[17] For *A. baumannii* and *P. aeruginosa*, breakpoints of *Enterobacterales* were used for interpretation of susceptibility testing for the antibiotics with no current CLSI/EUCAST breakpoints.

Colistin resistance was assessed using broth microdilution method according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines.[18] *Escherichia coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 were used as quality control strains.

The Modified Carbapenem Inactivation Method (mCIM) was performed to confirm carbapenemase production among resistant *K. pneumoniae* and *P. aeruginosa* isolates following the CLSI guidelines.[16] *Escherichia coli* ATCC 25922 was used for quality control purposes.

2.3. Molecular identification of antibiotic resistance genes

Bacterial DNA was prepared by boiling bacterial cultures in 200 µl of sterile distilled water for 10 min, followed by centrifugation for 10 min at 12,000 rpm. A volume of 2 µl of the supernatant was used as a template for each 20 µl PCR mixture. Different PCR protocols and primers were used (Table S1) to detect specific genetic determinants using Applied Biosystems SimpliAmp Thermal Cycler (ThermoFisher Scientific, USA).

Genetic screening included beta-lactamase encoding genes (bla_{SHV}, bla_{TEM}, bla_{GES}, bla_{CTX-M}, bla_{PER}, and bla_{VEB}),[19–21] carbapenemase genes (bla_{KPC}, bla_{VIM}, bla_{IMP}, bla_{NDM}, and $bla_{OXA-48-like}$),[22–23] quinolone resistance genes (*qnrA*, *qnrB*, and *qnrS*),[23–25] and tetracycline resistance genes (*tetA* and *tetB*).[26] Molecular screening for aminoglycosides included detection of aminoglycoside-modifying enzymes (AMEs) [*aad*A, *ant(2′′)-la*, *aph(3′)-VI*, *aac(3)-Ia*,aac(3)-lia, *aac(6′)-lh*, *aph(3′)-Ia*, and *aac(6′)-Ib*][27–29] and 16S rRNA methylases (RMTase); *armA*, *rmtA*, and *rmtB*.[30]

2.4. Detection of integrons and associated gene cassettes

Detecting the integrase gene, primers *intI1*,[31] *intI2*,[32] and *intI3*[33] were used for classes 1, 2, and 3, respectively. Associated gene cassettes were amplified for Class 1 and 2 integrons (Table S1). Primers 5′CS and 3′CS were used for amplification of the gene cassette associated with class 1 integron.[30] For Class 2 integron, hep74 and hep51 were used for amplification of the cassettes.[34,35] All amplified products were purified from the agarose gel using QIA quick Gel Extraction Kit (Qiagen, USA). DNA was sequenced using ABI Prism 377 automated sequencer (Applied Biosystems, USA).

2.5. Statistical analysis

Differences in the occurrence of phenotypic resistance and different genetic determinants among the three bacterial species were tested using the Chi-square ($\chi 2$) test. Differences among means with $P < 0.05$ were considered statistically significant. The antibiotic resistance (AR) gene's presence and the AMR profile data were first transformed into binary data (0 and 1) where 0 refers to antibiotic susceptibility and AR gene absence, while 1 represents antibiotic resistance and AR gene presence. To calculate the correlation coefficients for the data, Pearson's correlation coefficients (r) were implemented by the R stats package version (3.6.2) using *cor* function, significance of the correlation data was then estimated using the *cor.test* function. Finally, only the significant correlations ($P < 0.05$) were demonstrated in a visualized correlation matrix using the R corrplot package version (0.92).[35] Heatmaps demonstrating the AR genes' presence and absence were generated using the python packages: Seaborn[36] and Matplotlib.[37]

3. Results

3.1. High prevalence of carbapenem resistance

Antibiotic susceptibility patterns are shown in Table 1. Almost all the tested isolates (98.19 %; 109/111) displayed MDR phenotypes. Overall, no statistical difference in resistance rates was observed between the three bacterial species.

Table 1
Antimicrobial Resistance Patterns of The Gram-Negative Isolates.

Antimicrobial class	Antibiotic	*K. pneumoniae*	*A.baumannii*	*P.aeruginosa*	Total Resistance	Total Sensitivity
Beta lactams Group						
	Imipenem	100 %	89.47 %	100 %	96.08 %	2.94 %
	Meropenem	97.83 %	93.33 %	95 %	95.49 %	2.7 %
	Ertapenem	97.83 %	97.73 %	100 %	98.18 %	1.82 %
	Amoxicillin-Clavulanic	100 %	100 %	100 %	100 %	0 %
	Ampicillin	100 %	100 %	100 %	100 %	0 %
	Ampicillin-Sulbactam	100 %	97.22 %	100 %	98.88 %	1.12 %
	Aztreonam	95.45 %	100 %	100 %	97.92 %	1.04 %
	Cefadroxil	100 %	100 %	100 %	100 %	0 %
	Cefepime	100 %	97.37 %	89.47 %	97.06 %	2.94 %
	Cefoperazone	100 %	100 %	94.74 %	98.85 %	1.15 %
	Cefoperazone-Sulbactam	100 %	97.22 %	94.74 %	97.7 %	2.29 %
	Cefotaxime	10 %	100 %	94.74 %	98.85 %	1.15 %
	Cefoxitin	100 %	100 %	100 %	100 %	0 %
	Ceftazidime	100 %	97.78 %	95 %	98.19 %	1.8 %
	Ceftriaxone	100 %	100 %	100 %	100 %	0 %
	Cefuroxime-Sodium	100 %	100 %	100 %	100 %	0 %
Aminoglycosides						
	Tobramycin	100 %	81.58 %	89.47 %	90.11 %	8.79 %
	Amikacin	79.55 %	83.33 %	89.47 %	82.83 %	13.13 %
	Gentamicin	71.74 %	80 %	90 %	78.38 %	16.22 %
Tetracyclines						
	Doxycycline	82.61 %	84.44 %	100 %	86.48 %	13.51 %
	Minocycline	70.97 %	24.32 %	93.75 %	54.76 %	38.09 %
Quinolones						
	Levofloxacin	97.83 %	100 %	100 %	99.09 %	0.9 %
	Ciprofloxacin	97.78 %	100 %	89.47 %	97.06 %	2.94 %
	Ofloxacin	96.88 %	100 %	100 %	98.85 %	1.15 %

A considerable prevalence of CR isolates was observed. The most detected resistance phenotypes were against ertapenem (98.18 %), followed by imipenem (96.08 %), and meropenem (95.5 %) (Table 1). High prevalence of carbapenemase production among *K. pneumoniae* isolates (86.9 %; 40/46) compared to *P. aeruginosa* (45 %; 9/20) was confirmed using mCIM. As expected, all screened isolates exhibited resistance to β-lactam antibiotics (Table 1).

Among fluoroquinolone and quinolone-resistant isolates, a high prevalence of resistance to levofloxacin, ofloxacin, and ciprofloxacin (99.09 %, 98.85 %, and 97.06 %, respectively). Moreover, screening for tetracycline-resistant isolates revealed higher resistance rates to doxycycline (86.49 %) than to minocycline (54.76 %). Among minocycline-resistant isolates, a lower resistance rate was observed among *A. baumannii* (24.32 %) compared to *P. aeruginosa* and *K. pneumoniae* (93.75 % and 70.97 %, respectively) isolates. While among aminoglycoside-resistant isolates, the most detected resistance phenotypes were against tobramycin followed by amikacin and gentamicin (90.11 %, 82.83 %, and 78.38 %, respectively).

Overall, eleven *K. pneumoniae* isolates (23.9 %) were confirmed to exhibit colistin resistance. None of the screened *P. aeruginosa* and *A. baumannii* showed phenotypic resistance to colistin.

3.2. Occurrence of various antimicrobial resistance genes

Frequencies of AMR determinants are summarized in Fig. 1. For carbapenemase encoding genes, bla_{NDM} was the most prevalent gene (60.36 %; 67/111), with the highest predominance among *K. pneumoniae* (91.3 %; 42/46; $P < 0.0001$) compared to *P. aeruginosa* and *A. baumannii* isolates (55 % and 31.3 %, respectively). The bla_{VIM} gene was detected in one *P. aeruginosa* isolate but absent in *K. pneumoniae* and *A. baumannii* isolates. Also, bla_{KPC} and bla_{IMP} was absent in all isolates. For Class D carbapenemase, $bla_{\text{OXA-48-like}}$ was present in 42.34 % (47/111) of the isolates, significantly ($P < 0.0001$) higher frequency in *K. pneumoniae* (93.48 %;43/46) compared to *P. aeruginosa* (15 %; 3/20) and *A. baumannii* (2.22 %; 1/45) isolates. Interestingly, 39 (84.78 %) *K. pneumoniae* isolates harbored two carbapenemase genes (bla_{NDM} and $bla_{\text{OXA-48-like}}$). Only one *A. baumannii* and one *P. aeruginosa* co-produced these two carbapenemase genes.

Detection of other *β*-lactamase-encoding genes revealed that bla_{SHV} was more significantly detected ($P < 0.0001$) among *K. pneumoniae* (97.8 %; 45/46) isolates. While was detected in one *P. aeruginosa* isolate but absent in *A. baumannii* isolates. Similarly, the bla_{TEM} gene was significantly more prevalent among *K. pneumoniae* (71.74 %; 33/46) compared to *A. baumannii* (51 %; 23/45) and *P. aeruginosa* isolates (5 %, 1/20).

Examination of other extended-spectrum *β*-lactam-encoding genes revealed that bla_{GES} gene was more prevalent among *A. baumannii* (60 %;27/45; $P < 0.0001$) compared to *P. aeruginosa* (15 %; 3/20) yet absent in *K. pneumoniae* isolates. Screening for $bla_{\text{CTX-M}}$ of groups 1, 2, and 9 revealed high prevalence rates among *K. pneumoniae* isolates (82.61 %, 28.26 %, and 54.35 %, respectively). For *P. aeruginosa,* only one isolate carried $bla_{\text{CTX-M}}$ Group1, and the other $bla_{\text{CTX-M}}$ groups were not detected in any of the isolates. Additionally, only $bla_{\text{CTX-M}}$ Group 9 was detected in four (8.9 %) *A. baumannii* isolates. The bla_{PER} gene was less prevalent (6.3 %), it was mainly detected in *A. baumannii* (13.3 %), while only found in one *P. aeruginosa* isolate, and absent in *K. pneumoniae.* None of the isolates harbored bla_{VEB}.

Among the genes encoding for the aminoglycoside modifying enzymes, *aph(3′)-Ia* was the most prevalent (81.98 %; 91/111) among tested isolates and was found in all *K. pneumoniae* and almost all (97.78 %; 44/45) *A. baumannii* isolates. Nonetheless, this gene was found in one *P. aeruginosa* isolate. The *aac(6′)-Ib* gene was found in 64.86 % (72/111) of isolates and was more prevalent in *K. pneumoniae* isolates (82.61 %; 38/46) compared to *A. baumannii* (57.78 %; 26/45) and *P. aeruginosa* (40 %; 8/20). The *aph(3′)-VI* was exclusively found in *A. baumannii* isolates (42.22 %;19/45). On the other hand, *ant (2′)-Ia* was exclusively present in half of screened *P. aeruginosa* isolates. A similar prevalence rate for *aadA* was also observed in half of the *P. aeruginosa* isolates while being only present in one *A. baumannii* and one *K. pneumoniae* isolate. A low prevalence rate was observed in

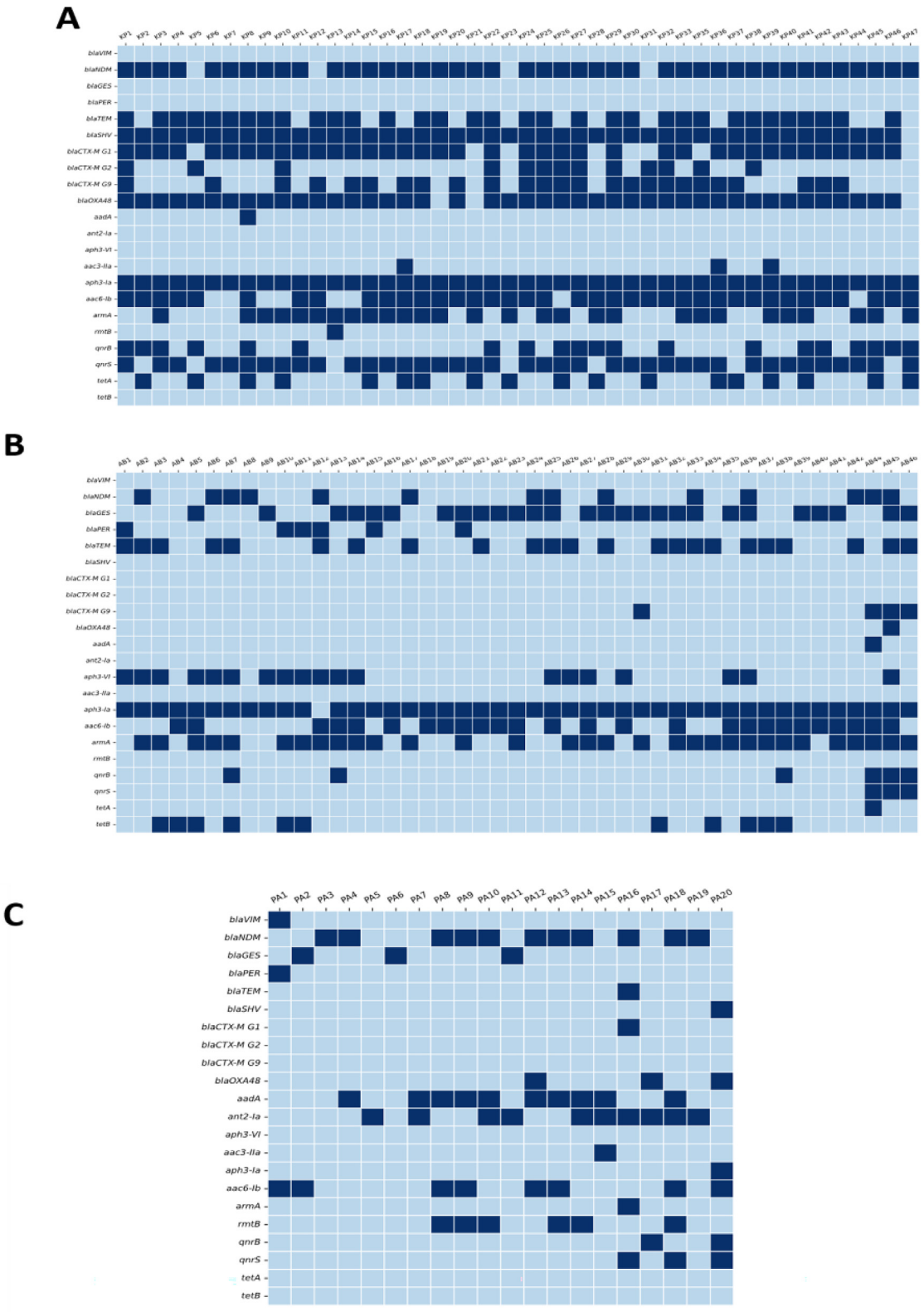

Fig. 1. The heat map shows the antibiotic resistance genes in the isolated Gram-negative bacteria (A: *K. pneumoniae*, B: *A. baumannii,* C: *P. aeruginosa.*). The dark blue color refers to the presence of the gene while the light blue one represents gene absence. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

*aac(3)-Iia (*3.6 %;4/111) which was present in three *K. pneumoniae* isolates and one *P. aeruginosa* isolate. All isolates were negative for *aac (3)-Ia* and *aac(6′)-Ih*. Molecular characterization of rRNA methyltransferases (RMTase) genes showed that the *armA* gene was more commonly detected in *K. pneumoniae* and *A. baumannii* (60.87 % and 68.89 %, respectively) compared to *P. aeruginosa* (5 %; 1/20). On

the other hand, *rmtB* was more prevalent in *P. aeruginosa* (30 %; 6/20) compared to *K. pneumoniae* (2.17 %; 1/46) while being absent in *A. baumannii.* None of the isolates harbored the *rmtA* gene.

Molecular characterization of plasmid-mediated quinolone resistance (PMQR) genes revealed that *qnrS* was significantly more prevalent among *K. pneumoniae* (84.78 %;39/46) compared to *P. aeruginosa* (15 % %;3/20) and *A. baumannii* (6.67 %;3/45) isolates. Similarly, *qnrB* was more prevalent among *K. pneumoniae* isolates (43.48 %;20/46) compared to *P. aeruginosa* and *A. baumannii* (10 %;2/20 and 13.33 %;6/45, respectively). The *qnrA* was absent in all isolates.

For tetracycline resistance genes, *tetA* was more prevalent among *K. pneumoniae* (18/46; 39.13 %) compared to *A. baumannii* isolates (2.22 %;1/45). None of *P. aeruginosa* isolates was found to harbor that gene. Moreover, *tetB* was only found in 11 *A. baumannii* isolates while being absent in the other bacterial species.

Although colistin resistance was detected in 23.9 % of *K. pneumoniae* isolates, none of them harbored the plasmid-mediated mobile colistin resistance (*mcr*) genes.

Characterization of different integron classes identified class 1 integron in 82 % (91/111) of screened isolates (89 %, 74 %, and 84 % of *K. pneumoniae, A. baumannii,* and *P. aeruginosa*, respectively). Class 2 integron was detected in only one *A. baumannii* isolate, while class 3 was absent in all bacterial isolates. Different gene cassettes were detected among different bacterial isolates (Fig. S1) including the dihydrofolate reductase: *dfrA*5 that was detected in *K. pneumoniae* conferring resistance to trimethoprim (GenBank accession numbers OQ401878, OQ401888, OQ401886, OQ401887, and OQ401889, Table S2). The aminoglycoside nucleotidyl transferase *aadA6* was detected in *K. pneumoniae* and *P. aeruginosa* conferring resistance to aminoglycosides (GenBank accession number OQ401879, Table S2). *A. baumannii* isolates carried two varying cassette arrays. The first array carried *aac(6′)-Ib3*, *aac(6′)-Ib-cr* and *cmlA5* gene cassettes, conferring resistance to quinolones, aminoglycosides, and amphenicols, respectively (GenBank numbers OQ401882 and OQ401883, Table S2). The second array carried *aRR-2* and *aadA1* gene cassettes, conferring resistance to rifamycins, aminocyclitols, and aminoglycosides (GenBank accession numbers OQ401884 and OQ401885, Table S2). Class 2 integron (identified in one *A. baumannii* isolate) harbored a cassette array carrying *dfrA*1, *SAT-2* and *ANT(3′)-IIa* genes coding for dihydrofolate reductase, streptothricin acetyltransferase, and aminoglycoside nucleotidyltransferase, respectively (GenBank accession numbers OQ401880 and OQ401881, Table S2).

3.3. Correlation between phenotypic and genotypic resistance patterns

The co-resistance pattern between different phenotypic and genotypic traits was determined by correlation analysis (Fig. 2). In *K. pneumoniae*, a positive correlation was detected between phenotypic resistance towards antibiotics of different classes such as between quinolones (ciprofloxacin and levofloxacin) and aminoglycosides (amikacin). Additionally, correlation analysis revealed the co-occurrence of different antibiotic-resistance genes. For instance, bla_{SHV} was found to coexist with $bla_{OXA\text{-}48\text{-}like}$. Likewise, bla_{NDM} coexisted with $bla_{CTX\text{-}M-1}$ (Fig. 2a).

In *A. baumannii* isolates, a positive correlation between phenotypic resistance to tobramycin and meropenem as well as ertapenem and between resistance to tobramycin and other β-lactamases. The coexistence between different antibiotic-resistance genes from different classes was also observed for bla_{TEM} and bla_{NDM} (Fig. 2b).

In *P. aeruginosa,* a positive correlation between carbapenems (meropenem), aminoglycosides (gentamicin, tobramycin, and amikacin), as well as cephalosporins (cefepime, and cefotaxime) was observed. Also, the coexistence of bla_{SHV} with *aph(3)la*, $bla_{OXA\text{-}48\text{-}like}$, and *qnrB* was detected (Fig. 2c).

4. Discussion

Recently, the Centre for disease control and prevention (CDC) stated that during the COVID-19 pandemic, there was substantial growth in some antimicrobial-resistant infections including CR *Acinetobacter*, ESBL-producing *Enterobacterales*, CR *Enterobacterales,* and MDR *P. aeruginosa*.[38] In Egypt, and other developing countries, the AMR crisis has been attributed to the overuse and misuse of antibiotics.

Almost all (98.2 %) of our screened isolates were MDR, a rate higher than any previous studies conducted in Egypt.[39–42] Moreover, 98.2 % of the tested isolates were found to be phenotypically resistant to carbapenems. This rate is alarmingly higher than former studies in Egypt.[43–44] This resistance against carbapenem poses a major concern since they are mainly used as the last line of treatment against drug-resistant pathogens.[45]

We further conducted a molecular screening for the five main carbapenemase encoding genes including bla_{KPC} (Amber class A carbapenemase gene), bla_{VIM}, bla_{IMP}, bla_{NDM} (Amber class B carbapenemase genes), and $bla_{OXA\text{-}48\text{-}like}$ (Amber class D carbapenemase gene). Co-production of different carbapenemases in a single isolate was observed among the three different bacterial species by molecular characterization of different carbapenemase encoding genes. The high prevalence of bla_{NDM} and $bla_{OXA\text{-}48\text{-}like}$ among Gram-negative isolates is consistent with previous results in Egypt supporting the endemicity of these genetic determinants among various clonal lineages in the North Africa region.[46] Conversely, the absence of bla_{KPC} and bla_{IMP} is consistent with studies describing the scarcity of those genetic determinants in the region.[44,47–50]

Colistin is broadly used as the last resort for the treatment of carbapenem-resistant Gram-negative infections. What makes the situation worse is the development of colistin resistance among *K. pneumoniae* in concordance with previous reports in Egypt.[51–52] Notably, the *mcr* gene was absent in all the isolates which might be attributed to other mechanisms of resistance.

To further investigate the role of MGEs in the elevated levels of AMR rates, different types of integrons were characterized. Class 1 Integron demonstrated widespread dissemination among most (82 %) of the isolates, regardless of the species. This represents a higher prevalence rate compared to previous studies[41,53] emphasizing the role of MGEs in AMR dissemination. Different gene cassettes were detected among different bacterial isolates and different integrin cassettes including resistance to different antibiotics including trimethoprim (*dfrA5*), aminoglycosides (*aadA1, aadA6, aac(6′)-Ib-cr),* quinolones (*aac(6′)-Ib3)*, and amphenicols (*cmlA5),* and rifamycin (*aRR-2)*. It is worth mentioning that *aac(6′)-Ib-cr*, previously reported in *Escherichia coli*,[54] is a mutated version of the 6′ aminoglycoside acetyltransferase-Ib gene, accordingly, the ability of the gene to act as a mobile cassette that can transfer from one bacterium to another confers a potential risk of the dissemination of this mutant. Although the integron does not carry genes for imipenem resistance, the association of rifampin resistance with imipenem resistance imposes a threat to combinational therapies used to treat infections with imipenem-resistant bacteria.[55–56] As previously reported,[57–61] class 2 integrons were scarcely detected being present in one *A. baumannii* isolate carrying a cassette array bearing *dfrA*1, *SAT-2* and *ANT(3′)-IIa* genes coding for dihydrofolate reductase, streptothricin acetyltransferase, and aminoglycoside nucleotidyl transferase, respectively. The integron array included cassettes commonly associated with class 2 integrons.[40] This study, and to the best of our knowledge, is the first study to report the presence of the *aadA6-orfD* in an array in Egypt, signifying the emergence and potential spread of an unfamiliar mutated version of genes coding for the AME. The *aadA6* gene is a version of the *aadA1* gene that shares about 70 % of DNA sequence similarity. The enzyme coded by *aadA6* also shares 75 % of the amino acids with enzymes encoded by *aadA1*, *aadA2*, and *aadA3*, while for the rest of the

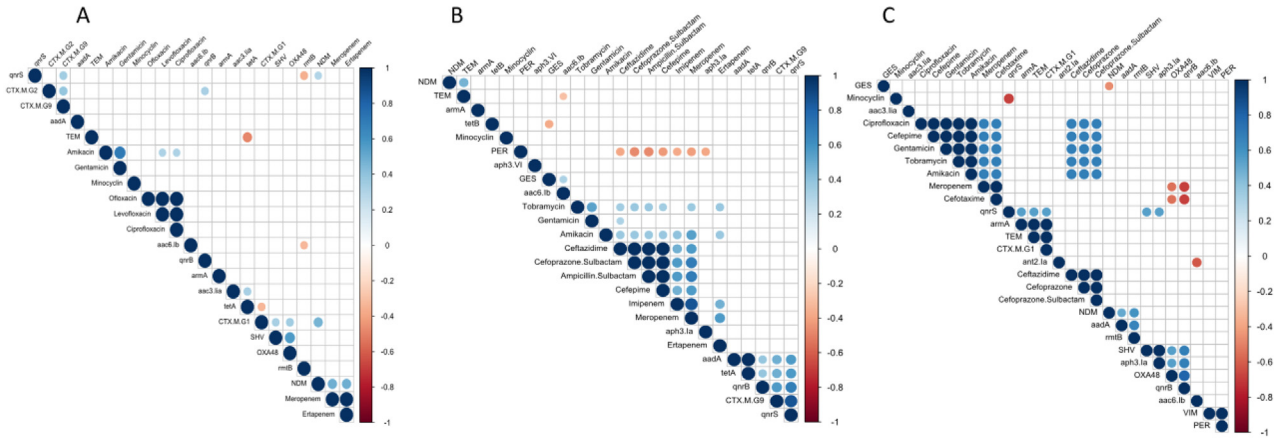

Fig. 2. Correlation matrix of antibiotic resistance phenotype and antibiotic resistance genotype characteristics demonstrating significant correlations ($p < 0.05$). White squares have no significant correlation. Blue dots denote are with a significantly positive correlation, whereas red dots demonstrate a significant negative correlation. The size of the dots and colour intensity corresponds to the value (r) of the correlation coefficient. A: *K. pneumoniae* correlation matrix, B: *A. baumannii,* C: *P. aeruginosa.* (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

mutated versions of the gene (*aadA4*, *aadA5*, and *aadAsc*), this enzyme shares about 60 % of amino acid similarity.[62] This gene was first identified in conjunction with an *orfD* gene in *P. aeruginosa* isolated from patients at a hospital located in France.[63] The protein product of *orfD* gene is currently of unknown function, however, it has been hypothesized that it is an open reading frame of function to the recombinational insertion of genes into the integrons.[64] Nevertheless, the *aadA6-orfD* integron array has been detected in *P. aeruginosa* samples from different countries worldwide.[64–67]

5. Conclusion

An alarming resistance rate among different Gram-negative pathogens to almost all available antibiotics and to last-resort antibiotics such as colistin and carbapenems constitutes a considerable burden.[68–69] This might be attributed to the extensive use of antibiotics in Egypt without prescription, especially during the COVID-19 pandemic. One of the limitations that we couldn't characterize novel AMR mechanisms, therefore future studies would implement whole genome sequencing among larger sample size of bacterial isolates, thus providing evidence-based stewardship strategies to reduce the burden of AMR in Egypt.

CRediT authorship contribution statement

Fatma A. Afify: Data curation, Formal analysis, Methodology, Writing – review & editing. **Ahmed H. Shata:** Investigation, Methodology, Writing – original draft, Writing – review & editing. **Nirmeen Aboelnaga:** Formal analysis, Methodology, Writing – original draft, Writing – review & editing. **Dina Osama:** Formal analysis, Visualization, Writing – original draft, Writing – review & editing. **Salma W. Elsayed:** Investigation, Methodology, Writing – original draft. **Nehal A. Saif:** Formal analysis, Investigation, Methodology, Writing – original draft. **Shaimaa F. Mouftah:** Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Sherine M. Shawky:** Investigation. **Ahmed A. Mohamed:** Investigation, Methodology, Writing – original draft, Writing – review & editing. **Omar Loay:** Formal analysis, Investigation, Methodology. **Mohamed Elhadidy:** Study design, Methedology, Investigation, Writting, review and editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jgeb.2024.100351

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