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Prevalence of Colistin resistance among difficult-to-treat Gram-negative nosocomial pathogens: An emerging clinical challenge

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ABSTRACT

Colistin, often referred to as a "salvation drug," is critical for treating severe infections caused by multidrug-resistant Gram-negative bacteria (MDRGNB), is experiencing an alarming increase in resistance worldwide. This study aims to elucidate the prevalence of colistin resistance across various drug-resistant phenotypes, specifically Usual drug-resistant (UDR) and Difficult-to-Treat (DTR), as well as among different antibiotic-class resistant categories, including Carbapenem-resistant (CR), Fluoroquinolone-resistant (FQR), and Extended-spectrum Cephalosporin-resistant (ECR) pathogens. Conducted across multiple healthcare settings in Egypt, we employed the Biomérieux VITEK® 2 system for isolate identification and selective media for screening colistin resistance. The minimum inhibitory concentration (MIC) of colistin was determined using broth microdilution (BMD), and PCR assays were conducted to detect plasmid-mediated mcr-1 and mcr-2 genes. Among 150 isolates, a concerning 78% demonstrated resistance to colistin, with significant occurrences in urinary tract infections (33%), respiratory tract infections (32%), and bloodstream infections (19%). The predominant colistin-resistant organisms identified included *Klebsiella pneumoniae*, *Escherichia coli*, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa*. Importantly, none of the isolates harbored the mcr-1 gene, and only one *E. coli* isolate harbored the mcr-2 gene. This research underscores the urgent need for enhanced surveillance and targeted therapeutic strategies, revealing the escalating incidence of colistin resistance among diverse drug-resistant Gram-negative pathogens, which presents a formidable challenge to effective clinical management.

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Introduction

Over the past 20 years, antimicrobial resistance (AMR) has been rapidly escalating globally, estimated to cause 700,000 deaths annually worldwide (Neill, 2014). AMR monitoring systems exist within and among antibiotic

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classes (Fridkin *et al.,* 2015). Several containment measures have been applied like the Standardization approaches of Multidrug resistance definitions. In 2012 Magiorakos *et al.* proposed three definitions: multidrug resistance (MDR), extensive drug resistance (XDR), and pan-drug resistance (PDR) but these definitions have not been consistently correlated with clinical outcomes. Subsequently, a clinically relevant categorization was proposed, including Usual Drug Resistance (UDR) by McDonnell *et al.* in 2016 and further refined by Kadri *et al.*, in 2018, emphasizing treatment-limiting resistance in Gram-negative bacteria (GNB).

The concept of Difficult-to-Treat Resistance (DTR) refers to cases where first-line agents, such as β-lactams and fluoroquinolones, are ineffective, often leading to reliance on "reserve" antibiotics that are less effective and carry higher risks. This classification is crucial for guiding empirical therapy and minimizing the threat posed by resistant pathogens.

Colistin, a cationic polypeptide antibiotic, is frequently utilized as a last-resort treatment for severe infections caused by MDR GNB (Zanichelli *et al.*, 2023). It acts by disrupting the outer membrane of Gram-negative bacteria, leading to cell lysis (Mohamed *et al.,* 2016), (Trimble & Mlyna, 2016). However, the increasing use of colistin, particularly in agriculture, has contributed to a troubling rise in colistin resistance (Col R) globally (Nation *et al.,* 2014) . This resistance is exacerbated by various mechanisms, including alterations in outer membrane porins and modifications to lipopolysaccharide, which reduce the drug's effectiveness (El-Sayed *et al.,* 2020). Notably, the discovery of plasmid-mediated colistin resistance genes (mcr), particularly mcr-1, has raised concerns about the rapid dissemination of resistance among various bacterial species (Liu *et al.*, 2016; Cabello *et al.,* 2017).

More than 27 different species of bacteria have been identified (Poirel, 2017; Andrade *et al.,* 2020) from six continents (Asia, Europe, Africa, North America, South America, and Oceania). Additionally, nine more *mcr* genes (*mcr*-2 to *mcr*-10) as well as their variants were reported. (Hussein *et al.,* 2021; Anyanwu *et al.,* 2020; Sharma *et al.,* 2022).

The purpose of the study is to estimate the true burden of colistin resistance (Col R) among drug-resistant phenotypes emphasizing the implications of emerging classification frameworks such as DTR and UDR. In addition to evaluating the dissemination of *mcr-1* and *mcr-2* in Gram-negative nosocomial pathogens, providing epidemiological references for decision-making in treatment and warranting routine screening for colistin resistance to guide appropriate therapy for future use.

Materials and Methods:

Isolates collection

Only non-duplicate Gram-negative nosocomial pathogens (GNB) were collected from Clinical microbiology labs of four hospitals, in Cairo, over 8 months. Species-level identification of isolates and antimicrobial susceptibility testing was carried out by Biomérieux VITEK® 2 system against 18 antibiotics. results were interpreted automatically according to CLSI guidelines (CLSI, 2023). The tested antibiotics were Ampicillin (AMP), ampicillin/clavulanic (AMC), piperacillin/tazobactam (TZP), Cefazolin (CZ), Cefoxitin (FOX), Ceftazidime (CAZ), Cefotaxime (CTX), Ceftriaxone (CRO), Ceftazidime/avibactam (CZA), Cefepime (CPM), imipenem (IPM), meropenem (MEM), gentamicin (GN), levofloxacin (LEV), ciprofloxacin (CIP), tigecycline (TGC) trimethoprim/sulfamethoxazole (SXT) and Colistin (COL). in addition to the rapid detection of Extended Spectrum Beta-Lactamase (ESBL) production which is based on the simultaneous assessment of the inhibitory effects of cefepime, cefotaxime, and ceftazidime, alone and in the presence of CA (clavulanate). Susceptibility results were interpreted according to CLSI recommendations: M07- A11 and M100-32th Edition 2023. (James *et al.*, 2023)

Phenotypic detection methods for Colistin resistance

The collected isolates were screened for colistin resistance using MacConkey agar (Condalab, Spain) containing colistin sulfate (Alpha chemika, India). Concentrations of colistin sulfate used varied according to the screened taxa, 4 µg/mL were used for *Enterobacteriaceae* and *A. baumannii members*, and 8 µg/mL for *P. aeruginosa* according to CLSI recommendations. The Colistin sensitivity test with Vitek 2® (bioMérieux) and broth micro-dilution assay confirmed screening results.

Minimum inhibitory concentrations (MIC) of colistin were determined by broth micro-dilution assay (BMD) and were performed according to the recommendations of the National Center for Disease Control (NCDC) (T, 2020). In brief, BMD was performed in sterile 96-well plates using two-fold serial dilutions of colistin sulfate and cation-adjusted Muller Hinton broth (CaMHB, Condalab, Spain). Colistin sulfate concentrations range from 2 to 1024 µg/mL. 50 µl of CaMHB broth was added to each well, then 25 µl of each Colistin dilution and 25 µl of bacterial suspension to each well with a final volume of 100 µl per well. The bacterial suspension was prepared by diluting 0.5 McFarland suspension 75 times to yield a bacterial concentration of approximately 5 x10^4 CFU/well. Positive control wells contained 25µl inoculum (Column 11) and 75 µl CaMBH. Negative control wells contained only 100 µl CaMBH (Column 12).–To determine MIC endpoints,

the lowest concentration showing no visual bacterial growth was recorded as the preliminary MIC value and further confirmed by turbidity measurement by ELISA reader (800TS microplate reader, Biotech) after 24 h incubation at OD 640 nm.

Phenotypic resistance categorization

In the current study, the isolates were classified into Susceptible (S), UDR, and DTR isolates. Susceptible isolates were described as sensitive to all antibiotics except those with intrinsic resistance. UDR was described as non-intrinsic in-vitro resistance or intermediate to one or more antibiotics but still effectively treated with first-line antibiotics (McDonnell *et al.*, 2016). Any GNB isolate exhibiting a non-intrinsic in-vitro intermediate or resistant phenotype to antibiotics belonging to carbapenem, beta-lactam, and fluoroquinolone categories was referred to as DTR. (Kadri *et al.*, 2018).

Additionally, phenotypic resistance was classified using the Centers for Disease Control and Prevention (CDC) surveillance classes (CDC, 2016) as CR, ECR, FQR, and beta-lactam/beta-lactamase inhibitor resistance (BL/BL-IR) were categorized under the European Committee for Antimicrobial Susceptibility Testing (EUCAST) criteria.

Genotypic detection of mobile Colistin resistance (MCR) genes

Preparation of DNA templates (Plasmid extraction):

Colistin-resistant Gram-negative bacteria (Col R GNB) DNA templates were prepared using Zyppy™ Plasmid Miniprep Kit (Zymo Research, USA) according to manufacturer recommendations. The extracted plasmids purity were assisted by a NanoDrop spectrophotometer (Thermo Scientific, UK). The ratio of absorbance at 260 and 280 nm of ∼1.8 is generally accepted as "pure" for DNA. (Garc *et al.*, 2020).

Polymerase chain reaction for mcr genes

Col R GNBs were screed for *mcr-1* and *mcr-2* genes using PCR according to Liu *et al.*, 2020 . PCR was performed in a 25 µL reaction mixture containing 12.5 µL of COSMO RED Master Mix, Willowfort), 2 µL of 20 pmol of each primer (Biosearch technologies, USA) 3 µl of 20 ng of each template DNA, and 5.5 µl nuclease-free water (Thermo Fisher Scientific, UK). PCR cycling conditions were denaturation at 94 °C for 2 min, 25 cycles of amplification at 94°C for 15 seconds and 55°C for 40 seconds, and a final extension step at 72°C for 1 min. The resulting DNA products were analyzed by gel electrophoresis system (Thermo Fisher Scientific, UK) in a 2% agarose gel at 90 V for 50 min. The primer sequences and the amplicon size of the genes are listed in Table 1.

Statistical analysis

Microsoft Excel v365 for Windows was used to compile the collected data, and the results were tallied and graphically represented. Frequency (count) and relative frequency (percentage) were used to portray categorical data.

Results

In this study, a total of 150 non-duplicate nosocomial GNBs were collected. According to the identification results, *K. pneumoniae* was the most prevalent accounting for 49 % (n=74), *E. coli* accounted for 23% (n=35), *Acinetobacter baumannii* accounted for 9% (n=14), *Pseudomonas aeruginosa* accounted for 8% (n=12) of isolates, *Proteus mirabilis*, *Serratia marcescens*, *Salmonella sp.*, *Citrobacter koseri*, and *Sphingomonas paucimobis* accounted for 5% (n=7), 2% (n=3), 2% (n=3), 1% (n=1) and 1% (n=1) respectively, as shown in Table 2.

Susceptibility testing revealed the resistance phenotypes were distributed as follows; 53 % (n=80) were UDR, 44% (n=66) were DTR, and only 3% (n=4) were susceptible to all antibiotics as shown in Figure 1. Antibiotic class categorical resistance was distributed as follows: 75% (n=113) were ECR, 63% (n=94) were FQR, and 49% were CR (n=74).

A. baumannii was the most prevalent with the DTR phenotype, accounting for 86% (n=12), followed by DTR *K. pneumoniae* accounted for 59% (n=44), Unlike the DTR phenotype, *P. mirabilis* and *E. coli* exhibited the highest UDR phenotype, accounting for 86% each.

Resistance to Reserve antibiotics results showed that 62% (n=93) were resistant to Ceftazidime-avibactam (CZA), 48% (n=42) were resistant to Tigecycline (TGC), out of which 8% (n=12) were of intrinsic resistance, and CoL R resistance rates represented 79% (n=118), out of which 8% (n=10) were of intrinsic resistance. The distribution of reserve antibiotic resistance across different species is shown in Table 2.

Although Urinary tract infections (UTI) were the most prevalent, accounting for 36% (n=54) of the total infections, DTR pathogens predominated Respiratory tract infections (RTI), with percentages of 59% (n=26), While Col R pathogens were predominant in UTIs with a percentage of 33% (n=36), as shown in Figure 2.

ICU infections accounted for 64% (n=96) of the total infections, out of which 56% (n=54) were DTR infections. Col R infections were mostly recovered 68% (n=63) from ICU.

Table 1. Primers used, expected amplicons, and sequences of mcr-1 and mcr-2 genes (Liu *et al.*, 2020)

Gene	Amplicons (bp)	Annealing temperature (°C)	Primer	Sequence*
mcr-1	205	55	*mcr*-1 Forward	TCCAAAATGCCCTACAGACC
			mcr-1 Reverse	GCCACCACAGGCAGTAAAAT
mcr-2	297		*mcr*-2 Forward	CCTTTTGTGCTGATGGGTTT
			mcr-2 Reverse	ATTTTGGAGCATGGTGGTGT

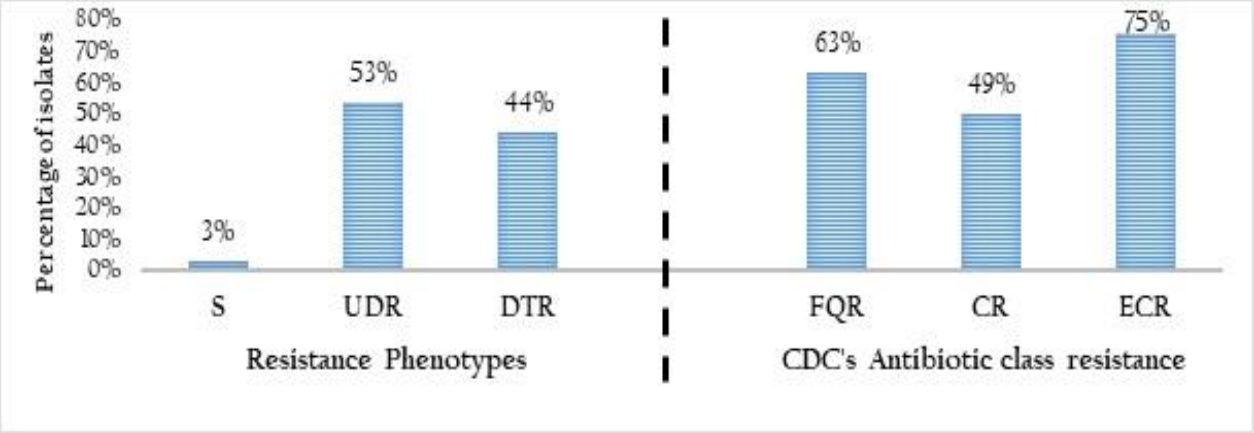

Fig. 1. Distribution of Resistance phenotypes and antibiotic categorical resistance of GNB in the study.

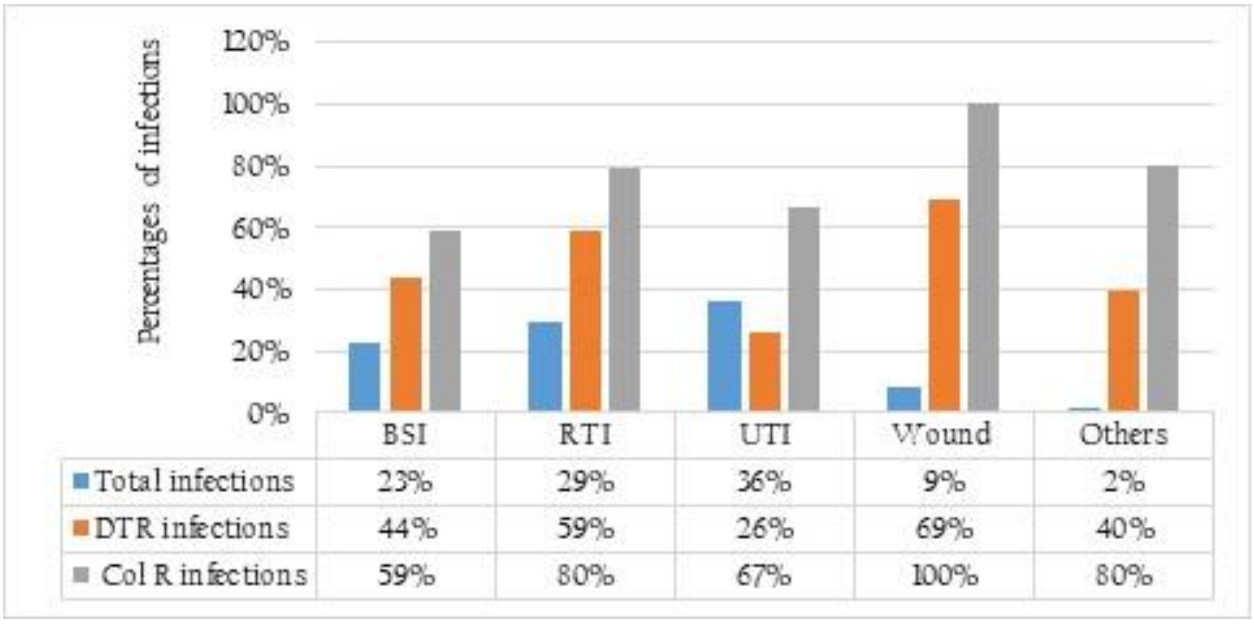

Fig. 2. Distribution of infections in the study.

BMD results showed that–colistin MIC ranges were 8->1024 µg/mL in *A. baumannii and E. coli*, 16->1024 µg/mL in *K. pneumoniae*, 512- 1024 µg/mL in *P. aeruginosa*, 1024 - >1024 µg/mL in *Salmonella sp.*, 16 µg/mL in *C. koseri* and >1024 µg/mL in *S. paucimobis* (Figure 3).

Table 2. Distribution of Resistance patterns among the Gram-negative pathogens.

Percentage of isolates	***A. baumannii* (n=14), N (%)**	***K. pneumoniae* (n=74), N (%)**	***E. coli* (n=35), N (%)**	***P. aeruginosa* (n=12), N (%)**	***P. mirabilis* (n=7), N (%)**	***Salmonella* sp (n=3), N (%)**	***S. marcescens* (n=3), N (%)**	***C. koseri* (n=1), N (%)**	***S. paucimobis* (n=1), N (%)**
	14 (10%)	74 (53%)	35 (25%)	12 (9%)	7 (5%)	3(2%)	3(2%)	1 (1%)	1 (1%)
Antibiotic class resistance									
CR	12 (86%)	46 (62%)	6 (17%)	6 (50%)	3 (43%)	0 (0%)	0 (0%)	0 (0%)	1 (100%)
ECR	12 (86%)	63 (85%)	26 (74%)	4 (33%)	3 (43%)	1 (33%)	0 (0%)	0 (0%)	1 (100%)
FQR	14 (100%)	54 (73%)	17 (49%)	5 (42%)	5 (71%)	0 (0%)	0 (0%)	0 (0%)	1 (100%)
BL/BL-IR	14 (100%)	59 (80%)	19(54%)	5 (42%)	2 (29%)	2(67%)	0 (0%)	0 (0%)	1 (100%)
New definitions									
S	0 (0%)	1 (1%)	1 (3%)	2 (17%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
UDR	2 (14%)	29 (39%)	30 (86%)	7 (58%)	5(86%)	3 (100%)	3 (100%)	1 (100%)	0 (0%)
DTR	12 (86%)	44 (59%)	4 (11%)	3 (25%)	2 (29%)	0 (0%)	0 (0%)	0 (0%)	1 (100%)
Reserve antibiotics resistance									
Col	12 (86%)	59 (80%)	23 (66%)	9 (75%)	7(100%)	3(100%)	0 (0%)	1(100%)	1(100%)
TGC	9 (64%)	21 (28%)	1(3%)	3 (25%)	4(57%)	0 (0%)	0 (0%)	0 (0%)	1(100%)
CZA	14 (100%)	56 (76%)	12 (34%)	6 (50%)	3 (43%)	1 (33%)	0 (0%)	0 (0%)	1(100%)

Abbreviations:
BL/BLIR, beta-lactam/beta-lactamase inhibitor resistance; CR, carbapenem resistance; DTR, difficult-to-treat resistance; UDR: Usual drug resistance; ECR, extended-spectrum cephalosporin resistance; FQR, fluoroquinolone resistance; MDR, multi-drug resistance; XDR, extensive drug resistance. Col: Colistin resistance; TGC: tigecycline resistance, CZA: Ceftazidime/avibactam.

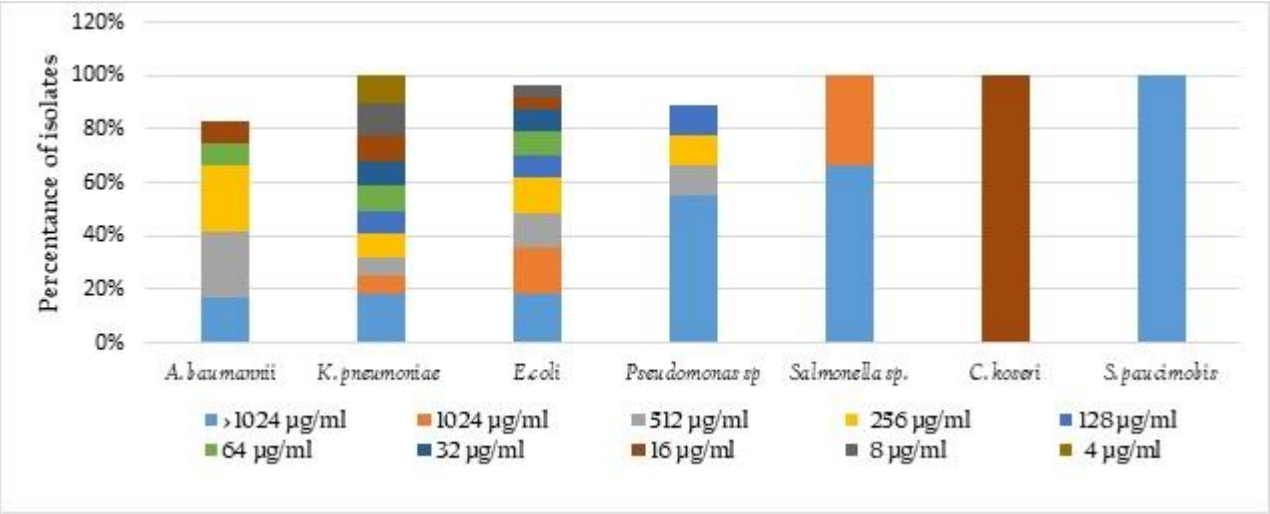

Fig. 3. Minimum inhibitory concentrations of Colistin in GNB pathogens in the study.

Susceptibility tests revealed that in Col R isolates 44% (n=48) were UDR, 48% (n=52) were DTR and 8% (n=8) were susceptible (S) to all antibiotics except for colistin.
The prevalence of Col R in GNB pathogens displaying DTR phenotypic patterns varies significantly among taxa. *A. baumannii* ranked as number one, with a prevalence DTR rate of 92% (n=11), followed by *K. pneumoniae* 82% (n=36), *E. coli* 75% (n=3), and *P. aeruginosa* 33% (n=1) Table 1. The ESBL phenotype tested positive in only *K. pneumoniae* and *E. coli*, representing a total of 25% (n=27) of the total Col R isolates, Col R /ESBL phenotype was reported in 24% (n=14) *K. pneumoniae* and 57% (n=13) *E. coli*. The coexistence of Col R with different Antibiotic-classes is illustrated in Figure 4

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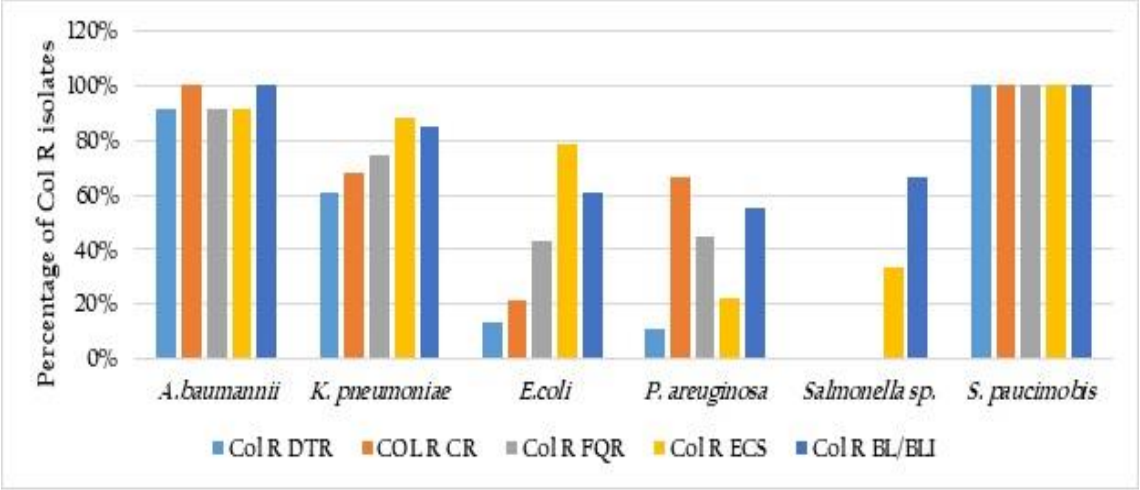

Fig. 4. Antibiotic class resistance among colistin-resistant isolates (coresistance).

Susceptibility of Col R pathogens to reserve antibiotics showed a resistance rate of ceftazidime/avibactam was 68% (n=73), while tigecycline resistance rates were 38% (n=41) as illustrated in Figure 5.

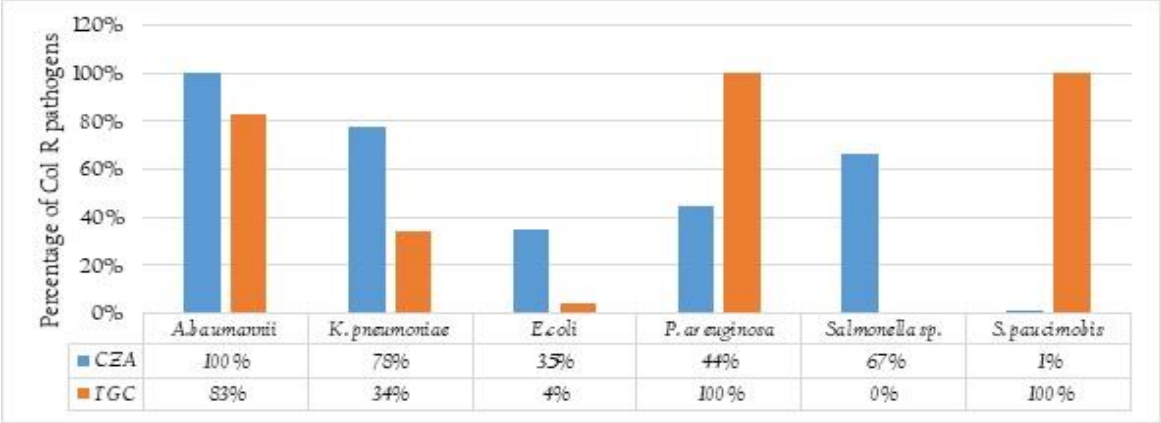

Fig. 5. The Susceptibility of Col R pathogens to reserve antibiotics; CZA: of ceftazidime/avibactam; TGC: tigecycline.

PCR results showed that only one *E. coli* was positive mcr-2 gene, Figure 6, and none of the isolates harbored the mcr-1 gene.

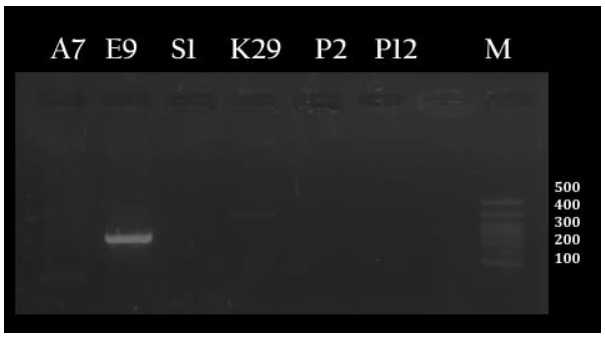

Fig. 6. *mcr*-2 gene PCR products showing positive *mcr*-2 gene (297 bp) in Lane E9 (*E. coli*), Corresponding to 300 bp marker band in lane M (ladder)

Discussion

Colistin is the last resort drug for the treatment of severe MDR infections. Col R increases the likelihood of excessive mortality among patients (Mantzarlis *et al.*, 2020; Papathanakos *et al.*, 2020). Based on the available data, Col R is more frequent in Southeast Asia and the Eastern Mediterranean region than in other parts of the world, with a global frequency of 11.2% (Germany 0.2%, United Kingdom 2.3%, India 8.2%, China 11.8%, and Lebanon 17.5%) (Pormohammad *et al.*, 2020).

Our findings demonstrate a striking 77% prevalence of non-intrinsic colistin resistance among nosocomial GNB, predominantly attributed to *Klebsiella pneumoniae* (55%), followed by *Escherichia coli* (20%), *Acinetobacter baumannii* (13%), and *Pseudomonas aeruginosa* (8%). These rates contrast sharply with those reported by Panigrahi *et al.*, who found an overall Col R prevalence of 19.6%, with *K. pneumoniae* at 9.2%. Discrepancies may arise from factors such as antibiotic misuse, varying infection control policies, and differences in sanitation practices across regions.

In a study conducted by Torres *et al*, BMD results showed that Col R GNB represented 57%, and was reported *in A. baumannii*, *K. pneumonia*, *E.coli*, and *P. aeruginosa* with Col R MIC range 4-32 µg/mL. Another study conducted another study from Egypt reported a MIC range of 4-32 µg/mL among *E.coli* and *K. pneumoniae* (Zafer *et al.*, 2019). Much higher MIC values were reported in the current study indicating the burden of colistin resistance in Cairo.

A. baumannii Col R were all among ICU patients and were prevalent in RTIs similar findings were reported, in which Col R *A. baumannii* dominated RTIs from Greece, Italy, and Spain (Nowak *et al.*, 2017).

The assessment of coexisting resistance with Col R showed that Col R/FQR and Col R/ECR represented 92% out of the total Col R *A. baumannii* isolates. In comparison, Col R/CR was reported in all of Col R *A. baumannii*. In a study conducted in Greece, Col R /CR phenotype was reported in 40% of *K. pneumoniae* and *A. baumannii* isolates (Falagas *et al.*, 2017). Our findings were consistent with a recent study by Biswas *et al*, in which all Col R *A. baumannii* isolates were ECR, and 87.5% were Col R/FQR. Moreover, the current study reported Col R/DTR *A. baumannii* isolate represented 92% (n=11).

The prevalence rates of DTR, FQR, ECR, and CR in *K. pneumoniae* were significantly higher than the monitoring results provided in China, the United States, and South Korea (Huh et al., 2020; Kadri et al., 2018; Zhang & Tian, 2022). Another study from India reported

a prevalence rate of 3.2% of DTR *K. pneumoniae* (Azam *et al.,* 2021).

Col R *K. pneumoniae* was predominant in ICUs and RTIs were the most frequent infection, These findings are inconsistent with Azam *et al.* in which UTIs were the most prevalent among Col R *K. pneumoniae*

In *P. aeruginosa* CR and FQR were the most frequent resistance phenotypes with a prevalence rate of 50% and 42% respectively. Meanwhile, DTR and ECR prevalence rates were 25% and 33% respectively. These findings were inconsistent with reports from China, the US, and South Korea, in which the reported prevalence rates were < 30% for all four resistance phenotypes (Huh *et al.,* 2020; Kadri *et al.,* 2018; Zhang *et al.,* 2022). Generally, the resistance of *P. aeruginosa* is low, earlier data from Europe revealed that Col R was < 0.5% (Kazmierczak et al., 2018), higher resistant rates, around 5-6% have been reported in Greece suggesting regional variations in susceptibility patterns.(Galani et al., 2020) (Pérez et al., 2019).

The most common phenotype of *E. coli* was ECR, with a prevalence rate of 74%, followed by FQR, with a prevalence rate of 49%. These findings contradicted monitoring data from the United States, South Korea, and China (Kadri *et al.*, 2018; Huh *et al.*, 2020; Zhang and Tian, 2022), which indicated that FQR was the most common phenotype in *E. coli, followed by ECR.* Moreover, prevalence rates *for DTR* and CR *E. coli* were much higher.

Col R *E. coli* represented 21%, and lower Col R prevalence rates were reported by (Biswas *et al.,* 2023). DTR *E. coli* were 4 and 3 exhibited the Col R/DTR resistance profile. Col R/ECR *E. coli* was the most predominant accounting for 78%, followed by 61% exhibited Col R/ Bl/Bl-IR, 43%) exhibited Col R/FQR and 22% exhibited Col R/CR phenotype. In a recent study, Col R *E.coli* was reported and exhibited 4 resistant phenotypes BL/BLIR, FQR, ECR, and CR (Biswas *et al.,* 2023).

Only one *E. coli* isolate was *mcr-2* harborer and none of the isolates were *mcr-1* carriers. This Col R *E. coli* had a MIC value of > 1024 µg/mL. These results were surprising because mcr-1 is more frequently reported in *E. coli* than mcr-2. Previous Egyptian investigations found mcr-1 in *K. pneumoniae* with a prevalence rate of 7.1% (Ibrahim *et al.,* 2021) and another investigation *mcr-1* gene in 2 isolates (4%), *E.coli* and *K. pneumoniae* with MIC > 16 µg/mL. while none of the isolates harbored the *mcr-2* gene.(El Sayed *et al.,* 2018).

A former study conducted in the Arabian Peninsula reported the *mcr-1* gene among MDR *E.coli* isolates (Sonnevend *et al.,* 2016). The investigation conducted by Torres *et al.* showed that *E.coli* isolates carried *mcr-1* and none of the isolates were *mcr*-2 carriers. The MIC range of the isolates was > 4-8 µg/mL. Other Genotypic research for plasmid-encoded *mcr* genes revealed that mcr-1 was reported in *E. coli* and *K. pneumoniae* isolates with a MIC value of 4 µg/mL while *mcr*-2 was not detected *(Zafer et al.,* 2019).

Earlier studies confirmed that MIC values >16 µg/mL in *E.coli* are not associated with plasmid-mediated *mcr*-genes, but rather with chromosomally mediated resistance (Torres *et al.,* 2022; Luo *et al.,* 2017;Sharma *et al.,* 2022).

S. paucimobilis was rarely reported in the clinical setting, particularly in immunocompromised patients. However, there are now quite a few case reports support evidence that implies this may be an emerging infectious pathogen worthy of further study and investigation (Sevtap *et al.,* 2018). As an emerging pathogen, *S. paucimobilis* needs to be treated with caution (Sevtap et al., 2018; Dsouza *et al.,* 2021). The isolated *S. paucimobilis* was DTR and Col R. Antimicrobial susceptibility variations have also been noted in numerous studies, where it was found that most strains were resistant to beta-lactam antibiotics while being susceptible to quinolones, carbapenems, beta-lactam/beta-lactamase inhibitors, and aminoglycosides (Lin *et al.,* 2010; Demir and Dadali, 2016). In research from Ghana, 90% of the *S. paucimobilis* isolates were MDR (Agyepong et al., 2018).,

Despite the reported resistance to Colistin, it is still successfully used in combination therapy as the salvation treatment for the lethal infections of MDR GNBs and Col R bacteria (Almutairi, 2022). Among these combinations were colistin with tigecycline, meropenem, gentamicin, or Fosfomycin (Dizbay *et al.,* 2010). Treatment for ventilator-associated pneumonia (VAP) caused by colistin-resistant bacteria with a combination of colistin, vancomycin, and rifampicin is successful (Tascini *et al.,* 2013).

Researchers suggested earlier that the available Col R infection treatment is tigecycline, and ceftazidime/avibactam. (Petrosillo *et al.,* 2019; Nusrat and Haque, 2020; De With *et al.,* 2016; Hawkey *et al.,* 2018), Unfortunately, our results show a high incidence of resistance towards ceftazidime/avibactam and a much lower resistance rate against tigecycline warranting caution in treatment selection.

Rapid Laboratory diagnosis plays a pivotal role in detecting colistin resistance, as it enables clinicians to tailor antibiotic therapies based on the susceptibility profiles of isolated pathogens. Furthermore, continuous monitoring of colistin resistance trends is essential for understanding the epidemiology of resistance within healthcare settings and the broader community. This ongoing surveillance allows for the early detection of emerging resistance patterns, which is critical for

implementing effective infection control measures and guiding public health policies.

Future work should be conducted on a larger number of GNB isolates. It should screen other *mcr* variants (*mcr*-3 to *mcr*-10) along with chromosomal mutations in genes involved in altering or losing Lipid A in LPS in infectious GN isolates. The most frequently reported colistin-related mutations are in genes encoding two-component systems (*pmrA/pmrB* and *phoP/ phoQ*) in several GNBs including *E. coli, K. pneumoniae, P. aeruginosa,* and *A. baumannii* (Laurent *et al.,* 2017). Additionally, evaluate the resistance rates of the recently launched therapeutic approaches for resistant GN infections.

Unfortunately, the available data on DTRs are mainly for assessment of BSIs; DTR can be used on any pathogens or any sites, this epidemiological tool should be updated with newly authorized drugs as it is a versatile phenotype that evolves with infections and our knowledge to fight them.

Even though Egypt has previously recognized shortcomings when it comes to surveillance and microbiology, The national healthcare-associated infections (HAI) monitoring program provides epidemiological information that will help infection control and preventative measures. (Talaat *et al.,* 2016) Additionally, unlike many other nations, Egypt has a nearly nonexistent infectious disease specialty, which hinders the creation and maintenance of antimicrobial surveillance systems, wise use of antimicrobials, and effective cross-specialist communication. Finally, different centers have varied procedures for gathering data, and there is no centralized organization in charge of planning data collection for AMR. Lack of community information-acquired MDR illnesses is another gap in Egypt's reporting of MDR GNIs (El-Kholy *et al.,* 2021). Future initiatives should focus on gathering more information about the molecular characterization of resistance genes in a wide range of healthcare facilities, such information would enable researchers to fully comprehend the country's MDR Gram-negative infection resistance trends. Future actions should also include national antimicrobial resistance control strategies, improved laboratory capabilities, and antimicrobial stewardship to regulate antibiotic usage.

Conclusion

This study provides an epidemiological snapshot that addresses critical gaps in understanding the Difficult-to-Treat phenotype and antibiotic-class resistance in Egypt. Our results reveal a concerning prevalence of reserve antibiotic resistance, particularly colistin, among nosocomial Gram-negative bacteria, with observed minimum inhibitory concentrations ranging from 8 to >1024 µg/mL. Importantly, we document the first occurrence of mcr-2 harboring *Escherichia coli* among nosocomial Gram-negative pathogens in Egypt, underscoring the urgent need for systematic monitoring of colistin resistance. Additionally, the emergence of DTR pathogens, such as *S. paucimobilis*, highlights the necessity for enhanced surveillance and strategic antibiotic stewardship. This research offers significant contributions to the understanding of antibiotic resistance dynamics in Egypt, emphasizing the need for proactive interventions to address the escalating threat of multidrug-resistant infections and to guide future clinical practices and public health initiatives.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable

Availability of data and material: the data supporting the findings of the study are available on request from the corresponding author, Hebatallah I. Abdelazeim Youssef.

Competing interests

The authors confirm that there is no conflict of interest.

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