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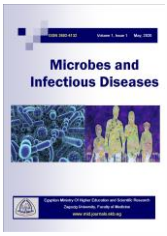

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Original article

Assessment of colistin resistance among nosocomial multidrug-resistant Gram-negative bacilli isolated from different clinical samples

Hanaa Fathy Abdel-Aty , Heba Mosaad El-Batal *, Noha Mahmoud Gohar

Department of Medical Microbiology and Immunology Department. Faculty of Medicine, Cairo University, Cairo, Egypt

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ABSTRACT

Background:
Multidrug resistance caused by Gram-negative pathogens is a significant global health concern. The increased resistance of these pathogens to commonly prescribed antibiotics has necessitated reintroducing colistin as the last treatment option. However, the uncontrolled consumption of colistin, particularly for multidrug-resistant (MDR) Gram-negative infections, has contributed to a surge in colistin resistance in many countries including Egypt. The present study aimed to determine colistin resistance among Gram-negative bacilli isolated from diverse clinical specimens. **Methods:** A total of 250 Gram-negative bacilli were included in the study. Antibiotic sensitivity for all isolates were performed using the Kirby-Bauer disc diffusion method. Colistin resistance was assessed by determination of minimal inhibitory concentration by broth microdilution method. **Results:** Out of 250 isolates, 36% were MDR, with *Escherichia coli* being the most predominant MDR isolates (68.4%), while 55.2% were extensively drug-resistant (XDR) with the predominance of *Acinetobacter baumannii* (71%). Resistance to colistin was reported in 22.8% of all studied isolates. Colistin resistance among MDR isolates was 10% and 30.4% among XDR isolates. The highest colistin resistance was observed among *Acinetobacter baumannii* (73.7%) followed by *Klebsiella pneumoniae* (12.3%), then *Pseudomonas aeruginosa*, and *Escherichia coli* (each 7%). The colistin-resistant isolates exhibited high resistance to ß-lactams antibiotics including 3rd and 4th generation cephalosporins (96.5%, 89.5% respectively), ciprofloxacin (80.7%), and aminoglycosides (71%). The isolates showed maximum sensitivity to doxycycline (58.5%), and sensitivity to imipenem and meropenem was 26%. **Conclusion:** High rates of MDR and XDR were observed among the recovered Gram-negative bacilli isolates. Colistin resistance was alarming in this study.

Introduction

For more than 50 years, antibiotic resistance has been increasing and spreading. It is currently among the most pressing public health issues of the twenty-first century [1]. The alarming rise in the global infection rates with resistant pathogens, especially Gram-negative bacilli (GNB), poses a serious threat to healthcare systems, delaying appropriate antibiotic therapy and raising mortality rates while also having negative impacts on the economy as a whole [2]. According to the World Health Organization, by 2025, 10 million deaths annually are predicted if current trends continue [3].

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* *Corresponding author:* Heba Mosaad El-Batal
E-mail address: *hebamosadelbatal@kasralainy.edu.eg*

The emergence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) bacterial strains and the lack of novel antibiotics pose a significant healthcare challenge [1].

Antibiotics such as carbapenems have long been regarded as the most potent broad-spectrum β-lactams against MDR GNB. Nevertheless, a growing number of research investigations have documented varied instances of carbapenem resistance *via* different pathways [4]. Consequently, colistin, an older-generation antimicrobial agent known for its high toxicity, presented as the last therapeutic alternative for potentially lethal infections caused by *Escherichia coli* (*E. coli*), *Klebsiella pneumoniae (K. pneumoniae*), *Acinetobacter baumannii* (*A. baumannii*) and *Pseudomonas aeruginosa* (*P. aeruginosa)* [5, 6].

Resistance to colistin has emerged globally in a number of nations across the world as a result of the increased usage of colistin as a therapy for MDR GNB infections. While colistin resistance generally represents less than 10%, colistin resistance rates continue to rise throughout the Mediterranean and Southeast Asia [7]. However, limited studies about colistin resistance have been published in Egypt. The objective of the current study was to assess colistin resistance among Gram-negative bacilli isolated from diverse clinical specimens and to investigate their antibiotic susceptibility profile and their association with colistin resistance.

Methods

Sample collection

This cross-sectional study included 250 Gram-negative bacilli isolates collected from Clinical Laboratories at Kasr Al-Ainy University Hospitals from August 2022 to February 2023. Organisms that were intrinsically resistant to colistin were excluded from our study. The study was done according to the Declaration of Helsinki and the protocol received approval from Cairo University's Faculty of Medicine Research Ethical Committee (approval number: N261-2023). The ethical committee waived the requirements for informed consent as the study was performed on bacterial isolates.

All isolates were subcultured on blood and MacConkey's agar (Oxoid, UK) aerobically at 37°C for 18-24 hours. Conventional biochemical reactions were used for routine identification of all GNB isolates [8]. Bacterial isolates that could not be definitively identified to the species level through conventional biochemical reactions underwent testing using MALDI-TOF MS (Bruker Daltonics, Billerica, MA, USA).

Antibiotic susceptibility testing

Kirby Bauer disc diffusion method

The Kirby-Bauer disc diffusion technique was used to evaluate the antibiotic susceptibility of all the bacterial isolates using commercially available antibiotic discs (Himedia, India), on Mueller-Hinton agar (Oxoid, UK) in accordance with CLSI standards. The following antibiotic discs were tested: amoxicillin-clavulanate (AMC), ampicillin-sulbactam (A/S), piperacillin-tazobactam (PIT), cefoxitin (CX), cefotaxime (CTX), ceftazidime (CAZ), cefepime (CPM), meropenem (MRP), imipenem (IPM), ertapenem (ETP), gentamycin (GEN), tobramycin (TOB), amikacin (AK), doxycycline (DO), ciprofloxacin (CIP), levofloxacin (LE) and trimethoprim-sulfamethoxazole (COT) [9]. *E. coli* ATCC 25922, and *P. aeruginosa* ATCC 27853 were used as standard control strains.

Multidrug-resistant isolates were those that exhibited acquired resistance to at least one agent in three or more antimicrobial categories, whereas XDR were those that exhibited resistance to at least one antibiotic agent in all categories except two or fewer [10].

Detection of colistin resistance among isolated GNB

Broth microdilution method for determination of MIC of colistin

The broth microdilution method (BMD) was used to determine the colistin minimal inhibitory concentration (MIC) of each GNB isolate with colistin sulfate powder (ADWIA Pharmaceuticals Co., Egypt) and cation-adjusted Muller Hinton Broth (CA-MHB) (Liofilchem, Italy). When the MIC of tested organisms, including Enterobacterales, *Acinetobacter species*, and *P. aeruginosa*, is equal to or higher than 4 µg/ml, the microbe is considered resistant to colistin [9]. *E. coli* carrying *mcr*-1 gene was used as a quality control strain.

Statistical analysis

The statistical software SPSS version 26 was utilized for data analysis. The qualitative data were expressed using percentages and frequencies. The Epi info statistical package was utilized to estimate the sample size. A total of 43 isolates were

determined to be necessary to generate a two-sided 90% confidence interval for a single proportion. The sample size was calculated using the large sample normal approximation and was extended by 10% based on the expected proportion of 0.196. To account for potential losses, the number of isolates was increased to 54, which was determined to be the minimum sample size required.

Results

The distribution of GNB isolates recovered from different clinical specimens (blood, body fluids, pus, sputum, tracheal aspirate, urine) is illustrated in **table (1).** The most frequently encountered organisms were *A. baumannii* (93 isolates, 37.2%), *K. pneumoniae* (70 isolates, 28%), *P. aeruginosa* (48 isolates, 19.2%), *E. coli* (38 isolates, 15.2%) followed by *Enterobacter cloacae (E. cloacae*) (1 isolate, 0.4%).

Antibiotic susceptibility profile of isolated Gram-negative bacilli:

The tested isolates exhibited variable degrees of susceptibilities against the tested antimicrobials as illustrated in **figure (1)**. *Enterobacter cloacae* isolate showed resistance to all tested antibiotics except carbapenems (imipenem, meropenem, and ertapenem), gentamicin, and amikacin.

Prevalence of MDR, XDR among the isolated GNB:

In the current study, out of the 250 isolates, 36% (90 isolates) were MDR, distributed as follows: *E. coli* (26/38, 68.4%), *K. pneumoniae* (34/70, 48.6%), *P. aeruginosa* (13/48, 27%), *A. baumannii* (17/93, 18.3%), while 55.2% (138 isolates) were XDR, distributed as follows: *A. baumannii* (66/93, 71%), *P. aeruginosa* (27/48, 56.25%), *K. pneumoniae* (34/70, 48.6%), *E. coli* (10/38, 26.3%) and *E. cloacae* isolate **(Figure 2)**.

Antibiotic susceptibility profile of MDR, XDR isolates:

Multi drug resistant *E. coli*, *K. pneumoniae*, and *P. aeruginosa* isolates displayed the lowest resistance to carbapenems and amikacin. Multi drug resistant *A. baumannii* isolates showed the lowest resistance to meropenem, and doxycycline **(Figure 3)**.

Regarding XDR isolates, XDR *E. coli* isolates showed the lowest resistance to carbapenems and aminoglycosides. XDR *K. pneumoniae* isolates displayed the lowest resistance to doxycycline, meropenem, and aminoglycosides. XDR *P. aeruginosa* isolates displayed the lowest resistance to carbapenems. XDR *A. baumannii* isolates showed the lowest resistance to doxycycline, co-trimoxazole, and ampicillin/sulbactam **(Figure 4)**.

Detection of colistin resistance among isolated GNB

In the present study, the MIC of colistin was evaluated by BMD to detect colistin resistance for 250 Gram-negative bacilli isolates. Fifty-seven isolates (22.8%) were resistant to colistin (57/250 isolates) and 77.2% were sensitive to colistin (193/250 isolates).

The highest colistin resistance was observed in *A. baumannii* 73.7% (42 isolates; 15 isolates were recovered from pus, 12 from sputum, 11 from blood, 2 from urine, and one isolate from each endotracheal and pleural fluid) followed by *K. pneumoniae* 12.3% (7 isolates; 4 isolates of them were recovered from pus, 2 from blood, and 1 from endotracheal aspirate) then *P. aeruginosa* 7% (4 isolates; all are isolated from pus specimens) and *E. coli* 7% (4 isolates, 3 isolates of them were collected from urine and one from pus specimens).

Distribution of colistin resistance among MDR and XDR isolates:

Out of the total 90 MDR isolates, 10% were colistin-resistant (9/90 isolates), while 90% were colistin-sensitive (81/90 isolates). Among the 138 XDR isolates, 30.4% were resistant (42/138 isolates) while 69.5 % (96/138 isolates) were sensitive to colistin.

Regarding colistin-resistant *E. coli* (no=4); 4 MDR isolates exhibited colistin resistance, and no colistin-resistant *E. coli*-XDR isolates. Regarding colistin-resistant *K. pneumoniae* (no=7); 2 MDR isolates were colistin-resistant, and 5 colistin-resistant isolates were discovered among XDR isolates. For colistin-resistant *P. aeruginosa* (no=4); one MDR isolate was colistin-resistant, 2 colistin-resistant isolates were discovered among XDR isolates, and one colistin-resistant isolate was sensitive to antibiotics (non-MDR/XDR). As regards colistin-resistant *A. baumannii* (no=42); 2 MDR isolates were colistin-resistant, 35 colistin-resistant isolates were discovered among XDR isolates, and 5 colistin-resistant isolates were non-MDR/XDR **(Figure 5)**.

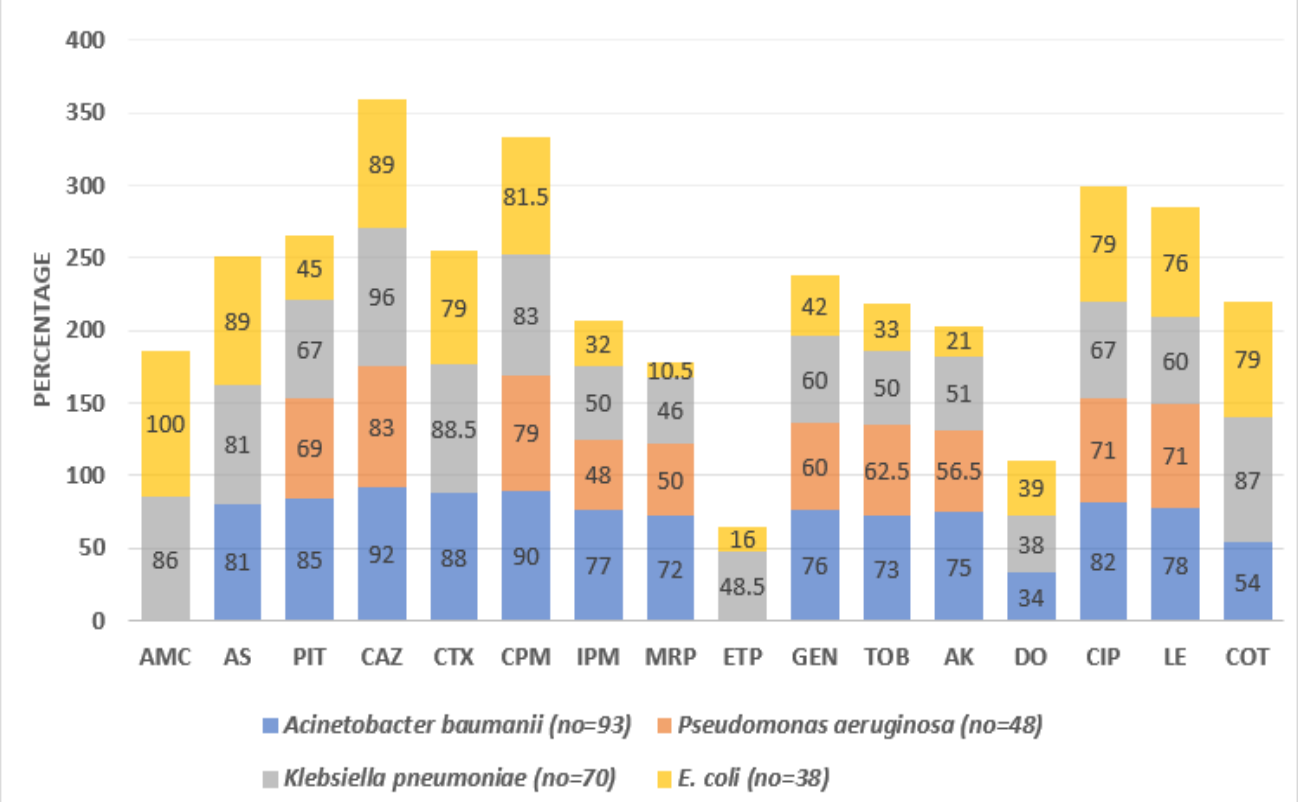
Antibiotic susceptibility profile of colistin-resistant isolates

The resistance pattern of colistin-resistant isolates is illustrated in **figure (6)**. All colistin-resistant *E. coli* isolates were sensitive to meropenem and ertapenem. Regarding colistin-resistant *K. pneumoniae* isolates, doxycycline was the most sensitive antibiotic followed by carbapenems, and aminoglycosides. The majority of isolates (75%) of *P. aeruginosa* resistant to colistin were sensitive to tobramycin. Regarding colistin-resistant *A. baumannii* isolates, 59.5% of isolates were sensitive to doxycycline.

Table 1. Distribution of GNB isolates recovered from different clinical specimens.

Organism / Specimen	*E. coli*	*K. pneumoniae*	*A. baumannii*	*P. aeruginosa*	*E. cloacae*	*Total*
Pus	20	38	35	31	1	125 (50%)
Urine	14	11	8	9	0	42 (16.8%)
Blood	3	8	22	2	0	35 (14%)
Sputum	0	7	24	4	0	35 (14%)
Tracheal aspirate	1	6	2	2	0	11 (4.4%)
Pleural fluid	0	0	1	0	0	1 (0.4%)
Ascitic fluid	0	0	1	0	0	1 (0.4%)
Total	38 (15.2%)	70 (28%)	93 (37.2%)	48 (19.2%)	1 (0.4%)	250 (100%)

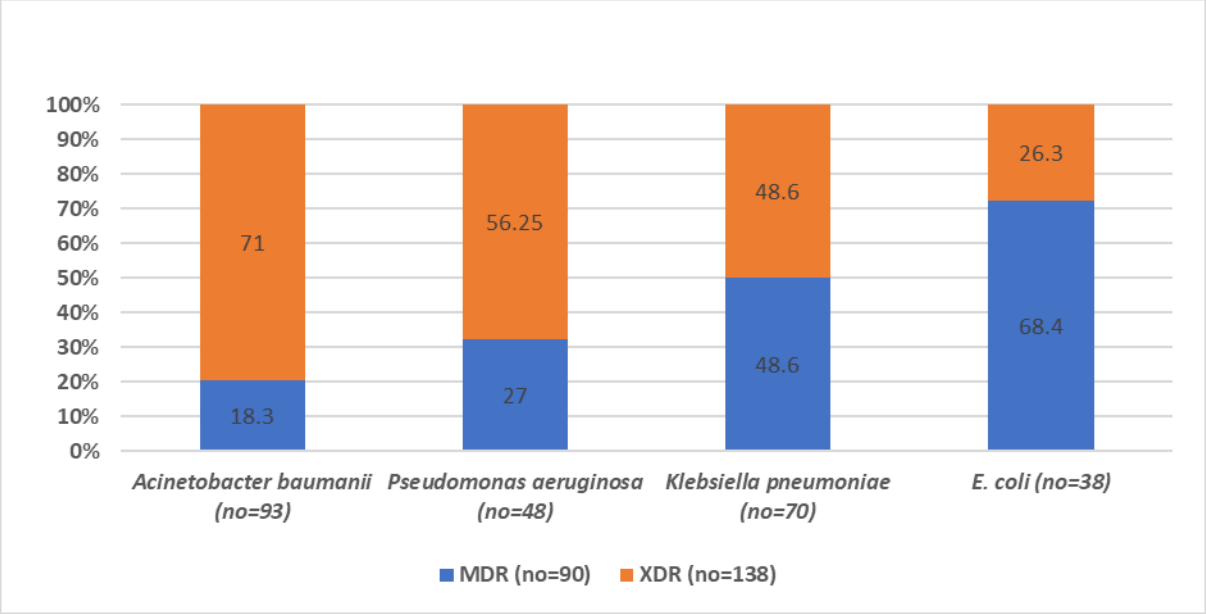
Figure 1. Resistance pattern of isolated Gram-negative bacilli.



Amoxicillin-clavulanate (AMC), ampicillin-sulbactam (A/S), piperacillin-tazobactam (PIT), cefoxitin (CX), cefotaxime (CTX), ceftazidime (CAZ), cefepime (CPM), meropenem (MRP), imipenem (IPM), ertapenem (ETP), gentamycin (GEN), tobramycin (TOB), amikacin (AK), doxycycline (DO), ciprofloxacin (CIP), levofloxacin (LE) and trimethoprim-sulfamethoxazole (COT).

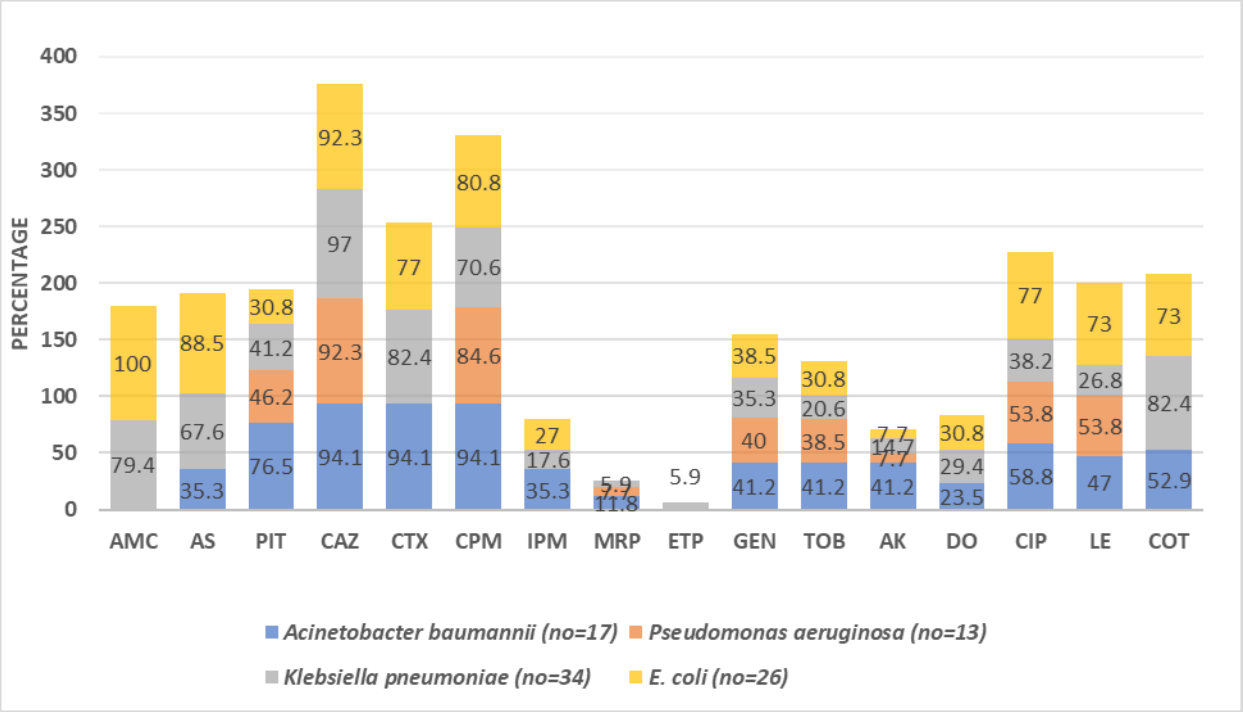
N.B: *Acinetobacter*, *Pseudomonas*, and *Klebsiella* species exhibit inherent resistance to certain antibiotics, so they weren't tested.

Figure 2. Percentage of MDR, XDR among Gram-negative bacilli isolates.



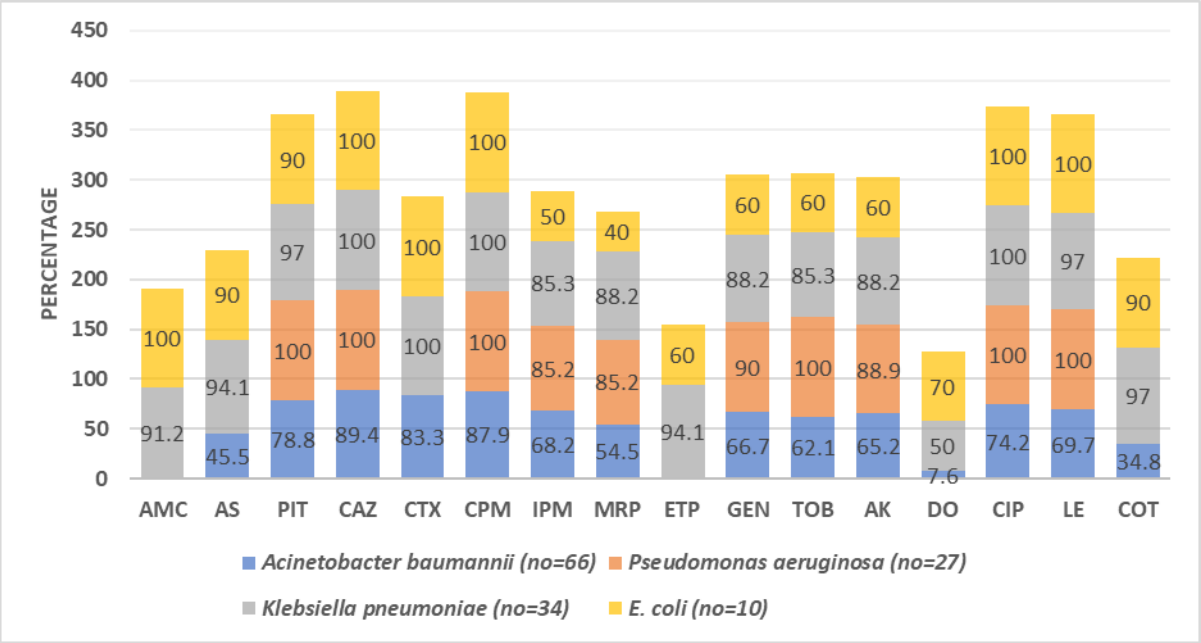
MDR (multi-drug resistant), XDR (extensively drug-resistant)

Figure 3. Percentage of resistance among MDR isolates.



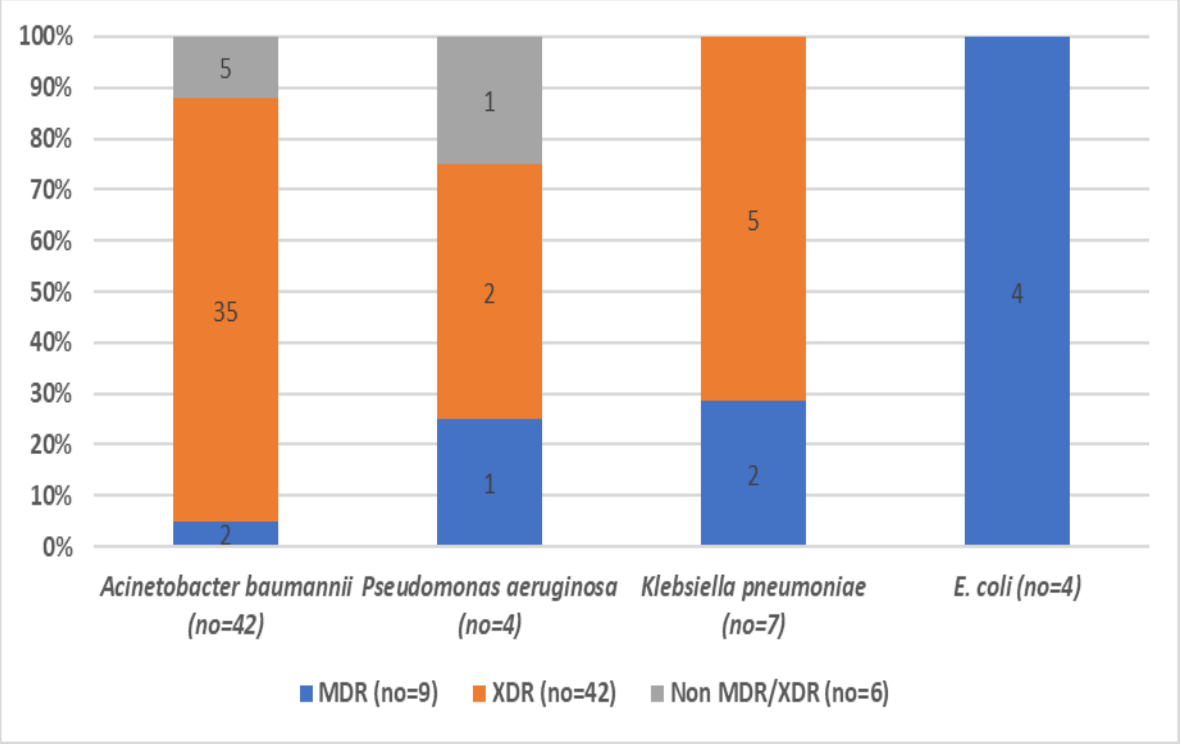
amoxicillin-clavulanate (AMC), ampicillin-sulbactam (A/S), piperacillin-tazobactam (PIT), cefoxitin (CX), cefotaxime (CTX), ceftazidime (CAZ), cefepime (CPM), meropenem (MRP), imipenem (IPM), ertapenem (ETP), gentamycin (GEN), tobramycin (TOB), amikacin (AK), doxycycline (DO), ciprofloxacin (CIP), levofloxacin (LE) and trimethoprim-sulfamethoxazole (COT).

Figure 4. Percentage of resistance among XDR isolates.



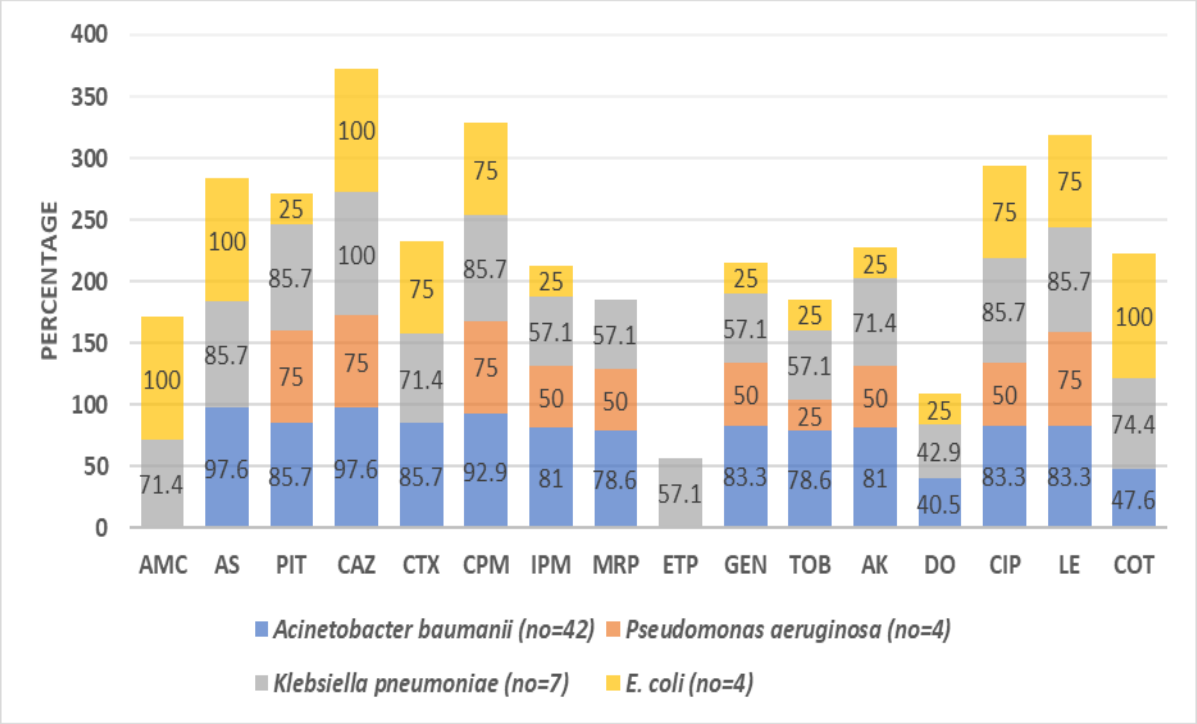
amoxicillin-clavulanate (AMC), ampicillin-sulbactam (A/S), piperacillin-tazobactam (PIT), cefoxitin (CX), cefotaxime (CTX), ceftazidime (CAZ), cefepime (CPM), meropenem (MRP), imipenem (IPM), ertapenem (ETP), gentamycin (GEN), tobramycin (TOB), amikacin (AK), doxycycline (DO), ciprofloxacin (CIP), levofloxacin (LE) and trimethoprim-sulfamethoxazole (COT)

Figure 5. Distribution of colistin resistance between MDR, XDR GNB



MDR (multi-drug resistant), XDR (extensively drug-resistant)

Figure 6. Resistance pattern of colistin-resistant isolates.



amoxicillin-clavulanate (AMC), ampicillin-sulbactam (A/S), piperacillin-tazobactam (PIT), cefoxitin (CX), cefotaxime (CTX), ceftazidime (CAZ), cefepime (CPM), meropenem (MRP), imipenem (IPM), ertapenem (ETP), gentamycin (GEN), tobramycin (TOB), amikacin (AK), doxycycline (DO), ciprofloxacin (CIP), levofloxacin (LE) and trimethoprim-sulfamethoxazole (COT)

Discussion

Antibiotic resistance has been highly prevalent in Egypt for more than 20 years, particularly among the Gram-negative bacteria that cause nosocomial outbreaks and infections [11]. According to an analysis of the epidemiology of MDR infections throughout the Arab League, Egypt reported higher resistance rates than its neighbors [12].

In the present study, 36% were categorized as MDR, while 55.2% were XDR out of 250 GNB isolates. Several studies reported similar rates of MDR among GNB isolates of 33.5%, and 40.5% [13, 14]. Our results were nearly in accordance with a study conducted at Cairo University Teaching Hospital's surgical intensive care unit, which found that 65% of GNB isolates exhibited an XDR pattern [15]. Other similar studies reported that 56.5% and 64% of the tested GNB isolates belonged to XDR category [16, 17].

On the other hand, higher rates of MDR and lower rates of XDR were recorded in several Egyptian studies; a study conducted at Tanta University Hospital found that 61.5% of GNB isolates were MDR, while 29.5% were XDR [18]. A study conducted at Alexandria University Hospital in Egypt observed that 55 % of GNB were MDR and 34.7% were XDR [19]. In Menoufia, 160 isolates of Enterobacterales were examined; 68.8% of them belonged to the MDR pattern and 25% were XDR [20].

According to a survey conducted by El-**Kholy et al.** [21], the MDR prevalence between various species of Gram-negative bacilli is high in Egypt, including 30-100% of *A. baumannii*, 21-100% of *P. aeruginosa*, 42.5-98.7% of *K. pneumoniae*, and 22.8-96% of *E. coli*. Excessive antibiotic consumption, use in non-human populations, and difficulties with infection prevention and management are assumed to be the causes of the high levels of resistance observed in Egypt.

In our study, *E. coli* was the most common MDR GNB, followed by *K. pneumoniae* These findings showed a strong correlation with previous studies indicating that *E. coli* and *K. pneumoniae* were the most common MDR bacteria [11, 13, 22].

Regarding XDR bacteria, we observed that *A. baumannii* had the highest prevalence (71%), followed by *P. aeruginosa* (56.25%), *K. pneumoniae* (48.6%), and *E. coli* (26.3%). These results were in agreement with a previous Egyptian study conducted at Cairo University Teaching Hospital in which 86% of *Acinetobacter spp.* were

XDR, followed by *Pseudomonas spp.* (63%), *Klebsiella species* (52%), and *E. coli* (47%) [15]. Several studies reported that XDR was higher among *Acinetobacter spp.* [14, 23, 24].

Colistin resistance among GNB in hospitals in Egypt has been a growing concern. In this work, the colistin resistance percentage between the studied isolates was 22.8%. This correlated well with several Egyptian studies in Minia and Assiut University Hospitals; where 23.1% and 20.8% *E. coli* strains were documented as resistant to colistin, respectively [25]. A study performed at the National Cancer Institute, found an increasing prevalence of colistin resistance among high-risk patients, with a 19.9% rate in 2022 compared to 8.8% in 2019 and 4.4% in 2016 in the same hospital [26].

Interestingly, a higher rate of colistin resistance was reported in a study carried out in the Gaza Strip which revealed that 41% of tested GNB isolates were resistant to colistin [27]. Another study reported that the colistin resistance among tested GNB was 63.4% [28].

According to several investigations carried out in different Egyptian hospitals; lower percentages of colistin resistance, ranging from 0 to 14%, were reported [29, 30-33]. In contrast to the rate found in our study, a lower rate of colistin resistance (10.4%) was noted in a prior investigation carried out in the same hospital (Cairo University Hospitals) in 2019 [34]. The increased resistance rate against colistin in our study could be explained by the widespread consumption of colistin in our hospital, particularly in high-risk patients who lack alternative treatment options. Additionally, the presence of co-morbidities among patients might have contributed to the higher resistance rate as well [35].

Globally, the incidence of Enterobacterales that are colistin-resistant rose from 2.6 to 3.6% between 2014 and 2019. This increase was seen in several regions, including Asia (3.3–6.7%), Latin America (2.7–4.3%), Europe (2.4–3.4%), Africa (2.1–2.6%), North America (1.2–2.6%), and Australia (0.6–2.7%) [36].

The variation in these findings can be attributed to several factors including; the differences in the populations studied, the misuse of colistin, different hospital policies for infection control, variations in sanitation practices, and the geographical distribution regions could influence the colistin resistance prevalence among Gram-negative bacilli [7].

In this work, *A. baumannii* exhibited the highest resistance towards colistin (73.7%), followed by *K. pneumoniae* (12.3%), then *E. coli* and *P. aeruginosa* (each 7%). Another study conducted in Egypt reported that *Acinetobacter species* were the most prevalent colistin-resistant isolates (14.3%) followed by *P. aeruginosa* (10.5%) [32].

Other studies showed that most *P. aeruginosa* isolates exhibited resistance to colistin [34,37]. Conversely, multiple studies have indicated that Enterobacterales exhibited a higher prevalence of colistin resistance [18, 33, 38-40].

The higher incidence of colistin resistance among *A. baumannii* isolates in our study is probably due to the high incidence of XDR *A. baumannii* strains as well as the total number of *A. baumannii* strains were higher than other GNB involved in this study.

In the current work, the colistin-resistant isolates displayed high resistance to ß-lactams including 3rd and 4th generation cephalosporins (96.5% and 89.5% respectively), piperacillin-tazobactam, fluoroquinolones (ciprofloxacin, 80.7%), and also to aminoglycosides (71%). The isolates showed maximum sensitivity against doxycycline (58.5%), and sensitivity to imipenem and meropenem was 26%.

Our results were correlated with **Shabban et al.** who found that 75% colistin-resistant isolates demonstrated a significant degree of resistance against cephalosporins, piperacillin-tazobactam, carbapenem, fluoroquinolones and aminoglycosides [32]. Another study recorded resistance to piperacillin-tazobactam and ciprofloxacin (90% each), carbapenems (30%), and aminoglycosides (20%) [26]. The variation in antibiotic policies implemented in healthcare settings across various geographical locations can account for the heterogeneity observed in the resistance pattern.

The potential treatment challenges with colistin-resistant infections face limited treatment options, emerging resistance to newer antibiotics, pharmacokinetic/pharmacodynamic challenges, the need for therapeutic drug monitoring, and the necessity of combination therapy.

Addressing colistin resistance requires comprehensive antimicrobial stewardship, infection control measures and surveillance, close monitoring

of colistin-resistant isolates, and containment measures, such as isolation and contact precautions, improving laboratory detection methods for colistin resistance. Global coordination and cooperation among countries, healthcare providers, and public health authorities are necessary to address the global spread of colistin-resistant bacteria and develop effective strategies for management and containment.

Limitations

This study has several limitations that should be taken into account. Firstly, the study was conducted in a single specialized medical center limiting the generalizability of the findings to the entire country. Secondly, patients' clinical history and risk factors were not included. It's important to know that resistance patterns can evolve over a longer timeframe, whereas this study had a limited duration. Therefore, the reported prevalence or incidence of antimicrobial resistance may not accurately reflect the overall situation. Additionally, due to financial constraints, molecular analyses to identify specific antibiotic resistance genes could not be conducted, which would have provided more comprehensive insights into the underlying mechanisms of resistance.

Conclusion

A significant proportion of MDR and XDR was found in the recovered isolates of Gram-negative bacilli. Our study has identified an increasing emergence of colistin resistance among GNB (22.8% overall), 10% among MDR isolates, and 30.4% among XDR isolates which were alarming findings. This highlights the importance of monitoring and controlling colistin resistance in hospitals in Egypt, through strict implementation of antibiotic policies and infection control measures to successfully prevent the spread of antibiotic resistance.

Conflict of interest

All authors affirm no conflict of interest in the work.

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Authors' contributions

All authors have substantially contributed to the conception and design, acquisition of data, data analysis, and interpretation. All authors have agreed on the content of the manuscript.

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