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ORIGINAL RESEARCH

Prevalence of multidrug-resistant Gram-negative pathogens isolated from febrile neutropenic cancer patients with bloodstream infections in Egypt and new synergistic antibiotic combinations

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Introduction: Bloodstream infections with multidrug-resistant (MDR) Gram-negative bacteria (GNB) are among the most frequent complications in immunocompromised cancer patients because of their considerable morbidity and mortality. Several guidelines on antimicrobial therapy have addressed empirical treatment for such serious infections; however, the emergence of microbial resistance has become a significant problem worldwide.

Materials and methods: In this study, starting from November 2015 to October 2016, a total of 529 blood specimens were collected from febrile neutropenic cancer patients at a tertiary care cancer hospital in Egypt.

Results: On examination for positive bacterial growth, it was found that 334 specimens showed no growth, while 195 were positive. Out of the 195 positive culture specimens, 102 (102/195, 52.3%) were Gram-negative and 93 (93/195, 47.7%) were Gram-positive. Out of the 102 GNB, 70 (70/102, 68.6%) were MDR, including *Escherichia coli* (27/70, 38.6%), *Klebsiella pneumoniae* (24/70, 34.3%), *Acinetobacter baumannii* (9/70, 12.8%), *Enterobacter cloacae* (4/70, 5.7%), *Pseudomonas aeruginosa* (2/70, 2.8%), *Klebsiella oxytoca* (2/70, 2.8%), and *Klebsiella ornithinolytica* (2/70, 2.8%). All MDR GNB showed high resistance to ampicillin, cefepime, ceftriaxone, and cephradine (minimum inhibitory concentration at which 50% of the isolates were inhibited [MIC_{50}] >512 µg/mL for each). However, they showed good susceptibility to colistin (MIC_{50} <1 µg/mL). The most common extended-spectrum β-lactamases (ESBLs) genes detected were *ctx-m* (39/70, 55.7%), *shv* (31/70, 44.3%), and *tem* (22/70, 31.4%). The most common aminoglycoside-resistant gene detected was *aac(6')-Ib* (42/70, 60%) followed by the plasmid-mediated quinolone resistance determinants; *qnrA* (2/70, 2.8%), *qnrB* (9/70, 12.8%), and *qnrS* (19/70, 27.1%). ESBL determinants were significantly associated with resistance to ciprofloxacin, levofloxacin, amikacin, and carbapenems (*P*-value <0.005). The fractional inhibitory concentration index for ampicillin/sulbactam plus ceftriaxone, ampicillin/sulbactam plus amikacin, and amikacin plus levofloxacin showed synergism against 29 (29/70, 41.4%), 19 (19/70, 27.1%), and 11 (11/70, 15.7%) isolates of the tested MDR GNB isolates, respectively.

Conclusion: Accordingly, new empirical antibiotics should be administered including the use of colistin or meropenem alone or both against the MDR GNB in neutropenic cancer patients.

Keywords: ESBLs, plasmid-mediated quinolone resistance, PMQR, fractional inhibitory concentration, FICI

Introduction

Bloodstream infections (BSIs) are defined as positive isolate(s) of blood culture and associated with clinical findings.[1] Cancer patients are among the key candidates

for this type of infections due to the methods of treatment they undergo, such as invasive surgery, chemotherapy, radiotherapy, immunosuppressive agents, or administration of anticancer drugs during hospital stay. Cancer patients sustaining BSI also have higher morbidity and mortality rates. Therefore, speedy identification of isolates, clinical diagnosis, and effective treatment of BSIs decrease the risk of mortality among cancer patients with BSI.[2] Infection with multidrug-resistant (MDR) pathogens including extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae are particularly prevalent among cancer patients.[3] Enterobacteriaceae cause approximately 65%–80% of documented Gram-negative infections in these patients. However, *Pseudomonas aeruginosa* is also associated with significant morbidity and mortality in immunocompromised hosts.[4] Resistance to the β-lactams is mediated primarily through the production of a variety of β-lactamases including Ambler class C β-lactamases and ESBLs. ESBLs are derived from older, plasmid-mediated hydrolyzing enzymes, primarily the TEM and SHV types (both are ESBL enzymes). Other antibiotic resistance coding genes (i.e., to fluoroquinolones [FQs], aminoglycosides, macrolides, carbapenems, etc.) on the same plasmid can confer a MDR phenotype in a subset of these pathogens.[5] Colistin resistance in Gram-negative bacteria (GNB) could be mainly attributed to excessive use of colistin in treating carbapenem-resistant bacteria.[6] Clinical studies strongly suggest that combination therapy is superior to monotherapy for carbapenemase-producing Enterobacteriaceae.[7–9] To improve potential survival among patients with high risk of death, combination therapy is recommended.[10] However, a combination of two or more active drugs (i.e., colistin, tigecycline, or fosfomycin) with carbapenem is associated with a better outcome.[11] Several antimicrobial guidelines have addressed empirical treatment for such serious infections; however, the rapid treatment of such infections has become a significant problem worldwide. Therefore, in this study, we aimed at evaluating the genetic bases of antimicrobial resistance of MDR GNB in cancer patients against the most commonly used antimicrobial agents used for the treatment of such infections. In addition, some antibiotic combinations have been evaluated for use against the clinically relevant MDR GNB pathogens recovered in our study.

Materials and methods

Specimen collections

Starting from November 2015 to October 2016, a total of 529 blood specimens were collected from 529 cancer patients with absolute neutrophils count <500/mm^3 and oral temperature >38°C over at least 1 hour from National Cancer Institute (NCI) Cairo University, Cairo, Egypt. The NCI is the largest tertiary cancer hospital in Egypt, drawing patients nationwide. The study was approved by the NCI Ethics Committee and Faculty of Pharmacy Ethical Committee Nr. 173, and written informed consent was obtained from either patients or parents of patients after explaining the study purpose. The collected blood was directly injected into Bactec® (Becton Dickinson, Franklin Lakes, NJ, USA) culture vials and incubated in the Bactec 9050® (Becton Dickinson) incubator. Positive blood culture specimens were directly streaked on blood agar, chocolate agar, and MacConkey agar (Oxoid, Cheshire, England) plates.

Identification of the recovered bacterial isolates

The recovered clinical isolates were categorized according to their Gram stain. Several biochemical tests were performed to identify different bacterial species. The biochemical tests used included triple sugar iron test, oxidase test, citrate utilization test, urease test, methyl red test, Voges–Proskauer test, and eosin methylene blue agar test; all said test media were produced by Oxoid. Identification was confirmed using Microscan® WalkAway-96 Plus auto identification system (Beckman Coulter, Miami, FL, USA).

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was carried out by both Kirby-Bauer disc diffusion method[12] using commercial discs (Oxoid) on Muller Hinton agar (Oxoid) at 37°C for 18 hours and minimum inhibitory concentration (MIC), which is defined as the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation.[13] MIC was carried out in triplicate and average MIC was calculated, as recommended by the Clinical and Laboratory Standards Institute (CLSI).[14] Isolates resistant to three or more classes of antimicrobials were considered as MDR isolates.[15] The tested agents used in Kirby-Bauer disc (μL/disc) diffusion method belonged to five different classes of antimicrobials: the aminoglycosides (amikacin 30 μg, gentamicin 10 μg, and tobramycin 10 μg), β-lactams (ampicillin 10 μg, amoxicillin/clavulanic acid 20 μg/10 μg, aztreonam 30 μg, cefotaxime 30 μg, ceftazidime 30 μg, cefoxitin 30 μg, ceftriaxone 30 μg, cefepime 30 μg, ampicillin/sulbactam 20 μg/10 μg, piperacillin/tazobactam 100 μg/10 μg, imipenem 10 μg, ertapenem 10 μg, cefazolin 30 μg, and meropenem 10 μg), FQs (ciprofloxacin 5 μg and levofloxacin 5 μg), antimetabolites (sulfonamides/trimethoprim

1.25 µg/23.75 µg), and polypeptides (colistin 10 µg). The reference strain *Escherichia coli* ATCC® 25922™ was used for quality control.

Combinations of antibiotics

The value of the fractional inhibitory concentration index (FICI) as a predictor of synergy has been investigated according to the protocol described by Hsieh et al,[16] where FICI = FIC of antibiotic A + FIC of antibiotic B. The FIC of antibiotic A = MIC of antibiotic A in combination/ MIC of antibiotic A alone. Moreover, FIC of antibiotic B = MIC of antibiotic B in combination/ MIC of antibiotic B alone. The result of FICI was interpreted as follows: FICI ≤0.5, >0.5–1, >1–4.0, and >4 indicate synergism, additive, indifference, and antagonistic effects, respectively. The term "synergism" indicates that the interaction between the two antibiotics causes the total effect of the antibiotics to be greater than the sum of the individual effects of each antibiotic. An "additive" term indicates that the final effect is equal to the sum of the effects of the two antibiotics. The term "indifference" means neither the antibiotics help nor hinder one another's activity. The term "antagonism" indicates both antibiotics' interactions cause decrease in the effects of one or both of the drugs.[17]

Plasmid DNA extraction from MDR isolates

Plasmid DNA extraction was done using QIAprep® Miniprep kit (Qiagen, Germantown, MD, USA) according to the manufacturer's specifications. The extracted plasmids were analyzed using agarose gel electrophoresis.[18]

Amplification of some resistance genes by polymerase chain reaction (PCR)

Amplification of the selected antibiotic resistance genes was carried out via PCR using the appropriate primers (Table 1). The plasmid DNA of the tested MDR bacterial isolates was used as template. Primers were synthesized by Invitrogen (Paisley, UK). Detection of the amplified products was done by agarose gel electrophoresis according to the protocol described by Sambrook and Russell,[18] and the expected size of DNA fragment was determined in comparison to DNA ladder (GeneRuler 100 bp or 1 kb Thermo Fisher Scientific, Waltham, MA, USA).

DNA sequencing of some selected PCR products

The PCR products were purified using GeneJET™ purification kit at Sigma Scientific Services Company (Cairo, Egypt). PCR products of some selected amplified genes of certain MDR GNB isolates were sent for sequencing at GATC (Konstanz, Germany) using ABI 3730 xl DNA sequencer (Thermo Fisher Scientific). The obtained sequence files were assembled into the final consensus sequence using Staden Package program version 3 (http://staden.sourceforge.net/).[19] The final assembled sequences were analyzed, annotated, and submitted into the NCBI GenBank database.

Statistical methods

Statistical analysis was done using SPSS version 22 (IBM., Armonk, NY, USA). Qualitative data were expressed as frequency and percentage. Chi-square and Fisher's exact

Table 1 Primer sequences, Ta, and expected product sizes for the tested genes

Gene	Primer	Primer sequence (5′ → 3′)	Expected product size (bp)	Ta (°C)	References
ctx-m	P_f	CGCTTTGCGATGTGCAG	550	47	Bonnet et al[20]
	P_r	ACCGCGATATCGTTGGT			
shv	P_f	GGTTATGCGTTATATTCGCC	867	47	Rasheed et al[21]
	P_r	TTAGCGTTGCCAGTGCTC			
tem	P_f	ATGAGTATTCAACATTTCCG	867		
	P_r	CTGACAGTTACCAATGCTTA			
aac(6′)-Ib aac(6′)-Ib-cr	P_f	TTGCGATGCTCTATGAGTGG	358	46	Hamed et al[22]
	P_r	CGTTTGGATCTTGGTGACCT			
qnrA	P_f	GCCCGCTTCTACAATCAAGT	347	60	
	P_r	GGCAGCACTATTACTCCCAAG			
qnrB	P_f	TATGGCTCTGGCACTCGTT	193		
	P_r	GCATCTTTCAGCATCGCAC			
qnrS	P_f	TCGGCACCACAACTTTTCAC	255		
	P_r	TCACACGCACGGAACTCTAT			

Notes: *ctx-m, shv,* and *tem* genes code for ESBLs; *aac(6')-Ib* gene codes for aminoglycoside 6'-N-acetyltransferase type Ib; *aac(6')-Ib-cr* gene codes for aminoglycoside 6'-N-acetyltransferase type Ib ciprofloxacin-resistant variant; *qnrA*, *qnrB*, and *qnrS* genes are PMQR determinants coding for quinolone resistance.
Abbreviations: Ta, calculated annealing temperature; ESBLs, extended-spectrum beta-lactamases; PMQR, plasmid-mediated quinolone resistance.

tests were used for comparisons of categorical variables. All tests were two-tailed, and *P*-value <0.05 was considered statistically significant.

Results

Patient characteristics

In the period from November 2015 to the end of October 2016, BSIs were detected in 102 of 529 (19.3%) febrile neutropenic patients. The patients' age ranged from 1 to 66 years, with a mean age of 16 years. There were 54 (54/102, 52.9%) males and 48 (48/102, 47%) females. Eighty-five (85/102, 83.3%) patients were diagnosed with hematological malignancies and 17 (17/102, 16.7%) with solid organ malignancies.

Antimicrobial susceptibility testing

A total of 529 blood specimens were collected of which only 195 specimens showed positive bacterial growth. Out of the 195 positive culture specimens, a total of 102 (102/195, 52.3%) were Gram-negative and 93 (93/195, 47.7%) were Gram-positive. The antibiotic susceptibility patterns of the Gram-negative isolates are shown in Table 2A and B. Out of the 102 GNB, 70 (70/102, 68.6%) isolates were resistant to three or more classes of antimicrobial agents and therefore considered MDR isolates. The MDR GNB include *E. coli* (27/70, 38.6%), *Klebsiella pneumoniae* (24/70, 34.3%), *Acinetobacter baumannii* (9/70, 12.8%), *Enterobacter cloacae* (4/70, 5.7%), *P. aeruginosa* (2/70, 2.8%), *Klebsiella oxytoca* (2/70, 2,8%), and *Klebsiella ornithinolytica* (2/70, 2.8%). The aforementioned MDR GNB isolates were selected for this study.

MIC against MDR Gram-negative isolates

All 27 MDR *E. coli* and 23 *K. pneumoniae* isolates had high MIC to ceftriaxone; therefore, they were considered as potential ESBL producers according to the CLSI guidelines.[14] Table 3 shows resistance profiles for MDR Enterobacteriaceae including *E. coli* (27/70, 38.6%), *K. pneumoniae* (24/70, 34.3%), *E. cloacae* (4/70, 5.7%), *K. oxytoca* (2/70, 2.8%), and *K. ornithinolytica* (2/70, 2.8%). Table 4 shows resistance profiles for non-fermenter MDR GNB isolates including *A. baumannii* (9/70, 12.8%) and *P. aeruginosa* (2/70, 2.8%). The MIC_{50} (MIC at which 50% of the isolates were inhibited) values of MDR *E. coli* and

Table 2A Antimicrobial susceptibility patterns of Enterobacteriaceae isolates

Antibiotic	Resistant isolates, n (%)					
	E. coli	*K. pneumoniae*	*K. oxytoca*	*E. cloacae*	*K. ornithinolytica*	*S. enterica*
	R	R	R	R	R	R
Ampicillin	34 (100)	32 (100)	3 (100)	9 (100)	2 (100)	0 (0)
Amoxicillin/clavulanic acid	31 (91)	28 (87)	2 (67)	9 (100)	0 (0)	0 (0)
Ampicillin/sulbactam	30 (88)	28 (87)	2 (67)	9 (100)	2 (100)	0 (0)
Piperacillin/tazobactam	26 (76)	26 (81)	2 (67)	3 (33)	0 (0)	0 (0)
Aztreonam	26 (76)	19 (59)	2 (67)	9 (100)	2 (100)	2 (100)
Meropenem	16 (47)	18 (56)	0 (0)	0 (0)	0 (0)	0 (0)
Imipenem	15 (44)	16 (50)	0 (0)	0 (0)	0 (0)	0 (0)
Cefoxitin	26 (76)	19 (59)	0 (0)	9 (100)	0 (0)	2 (100)
Cefepime	32 (94)	24 (75)	2 (67)	2 (22)	0 (0)	0 (0)
Ceftazidime	32 (94)	24 (75)	2 (67)	6 (67)	0 (0)	0 (0)
Cefotaxime	32 (94)	24 (75)	2 (67)	6 (67)	2 (100)	0 (0)
Ceftriaxone	32 (94)	24 (75)	2 (67)	5 (55)	2 (100)	0 (0)
Amikacin	10 (29.5)	14 (43)	0 (0)	2 (22)	0 (0)	2 (100)
Gentamicin	19 (56)	13 (40)	2 (67)	5 (55)	2 (100)	2 (100)
Tobramycin	18 (53)	19 (59)	2 (67)	2 (22)	0 (0)	2 (100)
Ciprofloxacin	27 (79)	21 (65)	2 (67)	3 (33)	2 (100)	0 (0)
Levofloxacin	27 (79)	18 (56)	2 (67)	0 (0)	2 (100)	0 (0)
Trimethoprim/sulfamethoxazole	32 (94)	25 (78)	2 (67)	9 (100)	2 (100)	0 (0)
Cefazolin	32 (94)	28 (87)	2 (67)	9 (100)	2 (100)	2 (100)
Ertapenem	16 (47)	19 (59)	0 (0)	0 (0)	0 (0)	0 (0)
Colistin	0 (0)	4 (12.5)	0 (0)	3 (33)	0 (0)	0 (0)
Total	34	32	3	9	2	2

Abbreviations: *E. coli*, *Escherichia coli*; *K. pneumoniae*, *Klebsiella pneumoniae*; *K. oxytoca*, *Klebsiella oxytoca*; *E. cloacae*, *Enterobacter cloacae*; *K. ornithinolytica*, *Klebsiella ornithinolytica*; *S. enterica*, *Salmonella enterica*; R, resistant.

Table 2B Antimicrobial susceptibility patterns of non-fermenter isolates

Antibiotic	Resistant isolates, n (%)		
	A. baumannii	*P. aeruginosa*	*S. maltophilia*
	R	R	R
Ampicillin	12 (100)	6 (100)	2 (100)
Amoxicillin/clavulanic acid	10 (83.4)	6 (100)	2 (100)
Ampicillin/sulbactam	6 (50)	6 (100)	2 (100)
Piperacillin/tazobactam	9 (75)	2 (33)	0 (0)
Aztreonam	8 (66.6)	3 (50)	2 (100)
Meropenem	9 (75)	0 (0)	2 (100)
Imipenem	10 (83.4)	0 (0)	2 (100)
Cefoxitin	12 (100)	6 (100)	2 (100)
Cefepime	12 (100)	3 (50)	2 (100)
Ceftazidime	10 (83.4)	2 (33)	0 (0)
Cefotaxime	10 (83.4)	6 (100)	2 (100)
Ceftriaxone	8 (66.6)	4 (67)	2 (100)
Amikacin	9 (75)	0 (0)	0 (0)
Gentamicin	9 (75)	2 (33)	0 (0)
Tobramycin	4 (33.4)	0 (0)	0 (0)
Ciprofloxacin	10 (83.4)	0 (0)	0 (0)
Levofloxacin	5 (41.6)	2 (33)	0 (0)
Trimethoprim/sulfamethoxazole	9 (75)	4 (67)	0 (0)
Cefazolin	12 (100)	6 (100)	2 (100)
Ertapenem	10 (83.4)	4 (67)	2 (100)
Colistin	2 (16.6)	2 (33)	0 (0)
Total	12	6	2

Abbreviations: *A. baumannii*, *Acinetobacter baumannii*; *P. aeruginosa*, *Pseudomonas aeruginosa*; *S. maltophilia*, *Stenotrophomonas maltophilia*; R, resistant.

K. pneumoniae are shown in Table 5. Overall, the MIC_{50} of ceftriaxone, cefepime, cephradine, or ampicillin was >512 µg/mL. Meropenem (4 µg/mL) and colistin (<1 µg/mL) had the lowest MIC_{50}.

Detection of resistance genes using PCR

Out of the 70 MDR isolates, 13 (13/70, 18.6%), 7 (7/70, 10%), and 3 (3/70, 4.3%) isolates carried *ctx-m*, *shv*, and *tem* genes, respectively. While 11 isolates (11/70, 15.7%) carried the three tested genes together, 10 isolates (10/70, 14.3%) carried *ctx-m* and *shv* genes, 5 isolates (5/70, 7.1%) carried *ctx-m* and *tem* genes, and 3 isolates (3/70, 4.3%) carried *shv* and *tem* genes. Therefore, the most prevalent ESBL gene was *ctx-m* (39/70, 55.7%), followed by *shv* (31/70, 44.3%) and *tem* (22/70, 31.4%), as shown in Figure 1. Forty-two out of 70 MDR isolates (42/70, 60%) carried the *aac(6')-Ib/aac(6')-Ib-cr* gene. Concerning plasmid-mediated quinolone-resistance (PMQR) determinants, *qnrS* was more predominant (19/70, 27.1%), followed by *qnrB* (9/70, 12.8%) and *qnrA* (2/70, 2.8%), as shown in Figure 1.

Nucleotide accession codes

The PCR products of some selected plasmid-mediated resistant genes of certain MDR GNB isolates were verified by DNA sequencing. The final consensus sequences were analyzed and submitted into the NCBI GenBank database under the accession codes KX580955, KX580956, KY612437, KY612438, KY612439, KY612440, and KY612441.

Genotypes of MDR GNB isolates

A total of 22 different genotypes were obtained based on the detection of antimicrobial resistant genes on the extracted plasmids of the respective MDR GNB isolates, as shown in Table 6. The obtained genotypes have been associated with different sensitivity profiles against 21 tested antimicrobial agents. Statistical analysis showed that there were significant associations between members of FQs (such as ciprofloxacin and levofloxacin) and the detection of *shv* and *aac(6')* genes; amikacin and the detection of *shv*, *tem*, and *aac(6')* genes; members of carbapenems (such as meropenem, imipenem, and ertapenem) and the detection of *shv*, *ctx-m*, and *aac(6')* genes (P-value <0.05), as shown in Table 6.

Combinations of antibiotics

All MDR GNB were tested against five different antibiotic combinations (ampicillin/sulbactam plus ceftriaxone, ampicillin/sulbactam plus amikacin, levofloxacin plus amikacin, ampicillin/sulbactam plus meropenem, and colistin plus meropenem) and the FICI was calculated for each combination. It was found that ampicillin/sulbactam plus ceftriaxone combination showed synergism against 29/70 (41.4%) isolates, while ampicillin/sulbactam plus amikacin combination showed synergism against 19/70 (27.1%) isolates. Tables 7–11 show the FICIs for five different antibiotic combinations for MDR *E. coli*, *K. pneumoniae*, *A. baumannii*, *E. cloacae*, and other MDR isolates, respectively.

Discussion

BSI remains a major cause of life-threatening complications in patients with cancer[23] and is directly associated with prolonged hospital stay, high health care costs, and increased risk of morbidity and mortality.[24,25] Bacteria are the most common cause of such infections.[26,27] Resistance to antibiotics is a persistent and a difficult clinical problem that is compounded by a shortage of new therapeutic compounds.[28] BSIs due to GNB are common in cancer patients during aggressive therapy.[4] Therefore, in this study, we evaluated both the phenotypic and genotypic bases of antimicrobial resistance of certain

Table 3 MIC (μg/mL) results for MDR Enterobacteriaceae isolates

	Number of isolates with MICs (μg/mL) indicated											
	<1	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**256**	**512**	**>512**
E. coli (n=27)												
SAM	0	0	0	0	0	0	R/1	R/6	R/1	R/3	R/4	R/12
AK	0	0	0	S/2	S/2	S/3	I/11	0	0	0	R/1	R/8
CE	0	0	0	0	0	0	0	0	0	0	R/5	R/22
CRO	0	0	0	0	0	0	0	0	0	0	R/10	R/17
FEP	0	0	0	0	0	0	0	0	0	0	R/4	R/23
MEM	S/3	S/9	0	R/3	R/3	R/3	R/2	R/2	R/2	0	0	0
AMP	0	0	0	0	0	0	0	0	0	0	R/2	R/25
LEV	0	0	0	I/1	0	0	0	R/2	R/3	R/5	R/8	R/8
CT	S/27	0	0	0	0	0	0	0	0	0	0	0
K. pneumoniae (n=24)												
SAM	0	0	0	0	0	0	R/4	0	R/3	R/2	R/6	R/9
AK	0	0	0	S/2	S/1	S/2	I/3	0	R/2	0	R/2	R/12
CE	0	0	0	0	0	0	0	0	0	0	0	R/24
CRO	0	S/2	0	0	0	0	0	0	0	0	R/7	R/15
FEP	0	0	0	0	0	0	0	0	R/1	R/2	0	R/21
MEM	S/7	S/4	I/2	R/3	R/1	R/2	R/2	0	R/1	R/2	0	0
AMP	0	0	0	0	0	0	0	0	0	0	R/2	R/22
LEV	0	0	S/5	0	R/2	0	R/2	R/3	R/1	R/2	R/3	R/6
CT	S/21	0	0	0	0	0	0	R/2	0	0	R/1	0
E. cloacae (n=4)												
SAM	0	0	0	0	0	I/1	R/3	0	0	0	0	0
AK	0	0	0	0	0	S/3	I/1	0	0	0	0	0
CE	0	0	0	0	0	0	0	0	0	0	0	R/4
CRO	0	S/3	0	0	0	R/1	0	0	0	0	0	0
FEP	0	0	0	0	0	0	0	0	0	0	R/1	R/3
MEM	S/4	0	0	0	0	0	0	0	0	0	0	0
AMP	0	0	0	0	0	0	0	0	0	0	0	R/4
LEV	0	0	S/3	0	R/1	0	0	0	0	0	0	0
CT	S/1	0	0	0	0	R/2	0	0	0	R/1	0	0
K. oxytoca (n=2)												
SAM	0	0	0	0	0	0	0	0	0	R/1	0	R/1
AK	0	0	0	0	0	S/2	0	0	0	0	0	0
CE	0	0	0	0	0	0	0	0	0	0	0	R/2
CRO	0	0	0	0	0	0	0	0	0	0	0	R/2
FEP	0	0	0	0	0	0	0	0	0	0	0	R/2
MEM	S/1	S/1	0	0	0	0	0	0	0	0	0	0
AMP	0	0	0	0	0	0	0	0	0	0	0	R/2
LEV	0	0	0	0	0	R/1	0	0	0	0	0	R/1
CT	S/2	0	0	0	0	0	0	0	0	0	0	0
K. ornithinolytica (n=2)												
SAM	0	0	0	0	0	0	R/1	0	0	0	0	R/1
AK	0	0	0	0	0	0	0	R/1	0	0	0	R/1
CE	0	0	0	0	0	0	0	0	0	0	0	R/2
CRO	0	0	0	0	0	0	0	0	0	0	R/1	R/1
FEP	0	0	0	0	0	0	0	0	0	0	R/1	R/1
MEM	S/1	S/1	0	0	0	0	0	0	0	0	0	0
AMP	0	0	0	0	0	0	0	0	0	0	R/1	R/1
LEV	0	0	0	I/2	0	0	0	0	0	0	0	0
CT	S/2	0	0	0	0	0	0	0	0	0	0	0

Abbreviations: MIC, minimum inhibitory concentration; MDR, multidrug resistant; *E. coli, Escherichia coli*; SAM, ampicillin/sulbactam; R, resistant; AK, amikacin; S, sensitive; I, intermediate; CE, cephradine; CRO, ceftriaxone; FEP, cefepime; MEM, meropenem; AMP, ampicillin; LEV, levofloxacin; CT, colistin; *K. pneumoniae*, *Klebsiella pneumoniae*; *E. cloacae*, *Enterobacter cloacae*; *K. oxytoca*, *Klebsiella oxytoca*; *K. ornithinolytica*, *Klebsiella ornithinolytica*.

Table 4 MIC (μg/mL) results for MDR non-fermenter isolates

	Number of isolates with MICs (μg/mL) indicated											
	<1	1	2	4	8	16	32	64	128	256	512	>512
A. baumannii (n=9)												
SAM	0	0	0	0	0	I/2	R/4	0	0	R/1	R/2	0
AK	0	0	0	0	0	0	0	0	0	R/2	R/3	R/4
CE	0	0	0	0	0	0	0	0	0	0	0	R/9
CRO	0	0	0	0	S/3	0	I/1	0	0	0	0	R/5
FEP	0	0	0	0	0	0	0	R/3	R/3	0	0	R/3
MEM	0	0	0	I/5	0	R/3	R/1	0	0	0	0	0
AMP	0	0	0	0	0	0	0	0	0	0	R/1	R/8
LEV	0	0	0	I/2	0	R/2	R/3	R/1	0	0	0	R/1
CT	S/7	0	0	0	0	0	0	R/2	0	0	0	0
P. aeruginosa (n=2)												
SAM	0	0	0	0	0	0	0	0	R/1	R/1	0	0
AK	0	0	0	0	0	0	0	0	0	0	0	R/2
CE	0	0	0	0	0	0	0	0	0	0	0	R/2
CRO	0	0	0	0	0	0	0	0	0	0	0	R/2
FEP	0	0	0	0	0	0	0	0	0	0	0	R/2
MEM	0	S/2	0	0	0	0	0	0	0	0	0	0
AMP	0	0	0	0	0	0	0	0	0	0	0	R/2
LEV	0	0	0	0	0	0	0	0	0	0	0	R/2
CT	0	0	0	0	0	R/1	R/1	0	0	0	0	0

Abbreviations: MIC, minimum inhibitory concentration; MDR, multidrug resistant; *A. baumannii*, *Acinetobacter baumannii*; SAM, ampicillin/sulbactam; I, intermediate; R, resistant; AK, amikacin; CE, cephradine; CRO, ceftriaxone; S, sensitive; FEP, cefepime; MEM, meropenem; AMP, ampicillin; LEV, levofloxacin; CT, colistin; *P. aeruginosa, Pseudomonas aeruginosa*.

Table 5 MIC_{50} (μg/mL) results for MDR isolates against some selected antimicrobial agents

MDR GNB (n)	SAM	AK	CE	CRO	FEP	MEM	AMP	LEV	CT
E. coli (27)	512	32	>512	>512	>512	4	>512	512	<1
K. pneumoniae (24)	512	>512	>512	>512	>512	4	>512	128	<1

Abbreviations: MIC_{50}, MIC at which 50% of the isolates were inhibited; MDR GNB, multidrug-resistant Gram-negative bacteria; SAM, ampicillin/sulbactam; AK, amikacin; CE, cephradine; CRO, ceftriaxone; FEP, cefepime; MEM, meropenem; AMP, ampicillin; LEV, levofloxacin; CT, colistin; *E. coli, Escherichia coli*; *K. pneumoniae*, *Klebsiella pneumoniae*.

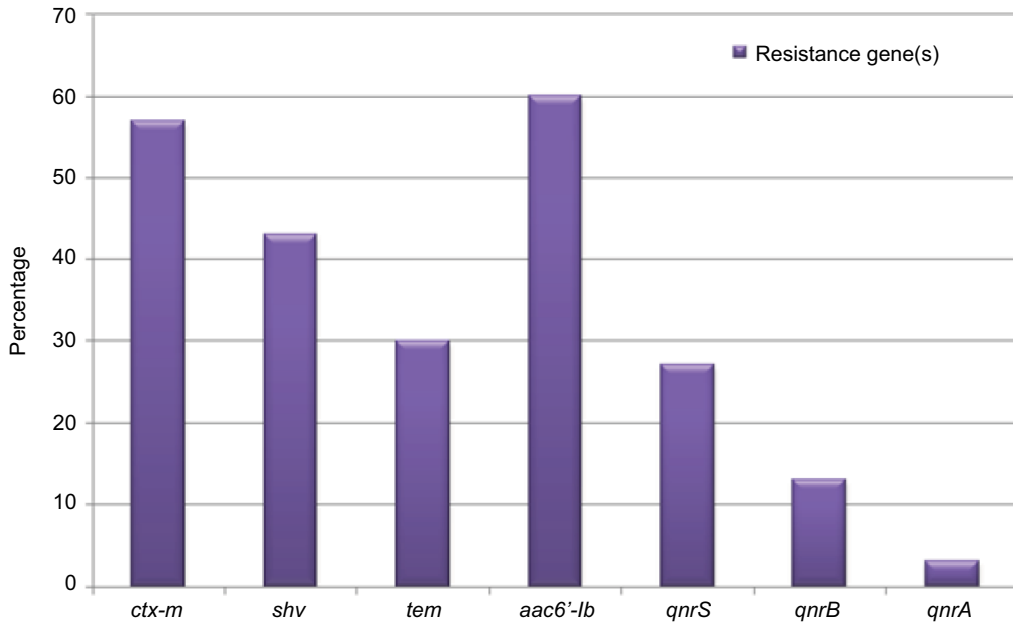

Figure 1 The prevalence of most common plasmid-mediated antimicrobial resistance genes among MDR GNB isolates.
Abbreviation: MDR GNB, multidrug-resistant Gram-negative bacteria.

Table 6 Percentage of different antimicrobial genotypes of MDR GNB isolates

MDR GNB	Genotypes	No. of isolates (%[a])	Significant associations[b]	Pearson chi-square
Positive (n=58)	*tem/shv/aac(6')-Ib*	2 (3%)	Ciprofloxacin/*shv*	0.022
	tem/shv/ctx-m/qnrS/aac(6')-Ib	3 (5%)	Ciprofloxacin/*aac-6'*	0.023
	tem/shv/ctx-m/qnrB/aac(6')-Ib	2 (3%)	Levofloxacin/*shv*	0.05
	tem/shv/ctx-m/qnrB/qnrS/aac(6')-Ib	2 (3%)	Levofloxacin/*aac-6'*	0.03
	tem/shv/ctx-m/qnrA/qnrS/a aac(6')Ib	2 (3%)	Amikacin/*tem*	0.026
	tem/shv/qnrS/aac(6')-Ib	2 (3%)	Amikacin/*shv*	0.008
	tem/ctx-m	4 (7%)	Amikacin/*aac-6'*	0.031
	tem/aac(6')-Ib	2 (3%)	Imipenem/*shv*	0.037
	tem/shv/ctx-m/aac(6')-Ib	3 (5%)	Imipenem/*ctx*-m	0.022
	shv/ctx-m	3 (5%)	Meropenem/*shv*	0.037
	shv	2 (3%)	Meropenem/*ctx-m*	0.004
	shv/aac(6')-Ib	2 (3%)	meropenem/*aac-6'*	0.05
	shv/qnrS/aac(6')-Ib	3 (5%)	ertapenem/*shv*	0.026
	shv/ctx-m/aac(6')-Ib	4 (7%)	ertapenem/*ctx-m*	0.004
	shv/ctx-m/qnrB/qnrS/aac-6'	1 (1.7%)		
	ctx-m	3 (5%)		
	ctx-m/aac(6')-Ib	6 (10%)		
	ctx-m/qnrS	2 (3%)		
	ctx-m/qnrB	2 (3%)		
	ctx-m/qnrB/qnrS/aac(6')-Ib	2 (3%)		
	qnrS/aac(6')-Ib	2 (3%)		
	aac(6')-Ib	4 (7%)		
Negative (n=12)	–	12 (17)		

Notes: Genotypes, plasmid-mediated antimicrobial resistance. [a]Percentages were calculated with reference to the number positive MDR GNB. [b]Significant association between antibiotic resistance and PCR detection of the respective gene on plasmids.
Abbreviations: MDR GNB, multidrug-resistant Gram-negative bacteria; PCR, polymerase chain reaction.

clinically relevant MDR GNB isolated from cancer patients against the most common antimicrobial agents used in treatment. In addition, the efficacy of some antibiotic combinations has been evaluated against such MDR GNB pathogens. In the present study, the frequency of Gram-negative isolates was slightly higher than that of Gram-positive isolates. On the contrary, another study conducted in Egypt reported that Gram-positive organisms were the predominant causative agents of BSI in cancer patients constituting about 72% compared to 24% of the Gram-negative organisms.[29] The results showed that the 34 *E. coli* isolates were resistant to ampicillin, while the most effective antimicrobial agents were colistin, amikacin, and imipenem followed by meropenem and ertapenem. Another study by Al-Mulla et al reported that *E. coli* isolates were highly susceptible to imipenem, meropenem, piperacillin/tazobactam, and amikacin, and less susceptible to gentamicin (55%) and ciprofloxacin (78%).[30] In the present study, *K. pneumoniae* isolates showed high susceptibility to colistin and were less susceptible to amikacin, imipenem, meropenem, and gentamicin. In another study conducted by Xiao et al, *K. pneumoniae* bloodstream isolates showed threatened resistance to the most of routine antibiotics and only meropenem, imipenem, amikacin, and piperacillin/tazobactam had relative low resistant rates.

In this study, 68.6% of isolates were resistant to three or more classes of antimicrobial agents and therefore considered MDR isolates. This finding agreed with the study conducted in Egypt by El-Mahallawy et al who reported that MDR was identified in 69% of bacteria isolated from positive blood cultures. This is due to the increasing use of chemotherapy and other immunosuppressive treatments, which result in more prevalent infections and reliance on antibiotic treatment, giving rise to a variety of resistant microbes.[32]

According to the 2017 guidelines on antimicrobial therapy, particularly the San Francisco Medical Center Guidelines, either cefepime or piperacillin/tazobactam is listed as an initial therapy in febrile neutropenic cancer patients.[33] While according to our findings, the recovered GNB including *E. coli*, *K. pneumoniae*, and *A. baumannii* showed higher resistance to these two antibiotics, which are listed in most guidelines (94%, 75%, and 100% resistance to cefepime and 76%, 81.25%, and 75% resistance to piperacillin/tazobactam

Table 7 FICI calculated for five different antibiotic combinations for MDR *E. coli* isolates

No.	SAM + CRO		SAM + AK		AK + LEV		SAM + MEM		CT + MEM	
	FICI	Interpretation	FICI	Interpretation	FICI	Interpretation	FICI	Interpretation	FICI	Interpretation
1	2	Indifference	2	Indifference	2.75	Indifference	1.5	Indifference	2	Indifference
2	1.67	Indifference	2.5	Indifference	2	Indifference	1.67	Indifference	2	Indifference
3	1.75	Indifference	1	Additive	2	Indifference	1.125	Indifference	1	Additive
4	0.5	Synergism	1.5	Indifference	1.37	Indifference	1	Additive	2	Indifference
5	2	Indifference	2	Indifference	1.5	Indifference	1.1	Indifference	1.1	Indifference
6	0.145	Synergism	0.145	Synergism	0.02	Synergism	1	Additive	2	Indifference
7	2	Indifference	0.11	Synergism	0.27	Synergism	0.53	Additive	1.167	Indifference
8	1.75	Indifference	2.6	Indifference	2	Indifference	1	Additive	2	Indifference
9	1	Additive	0.5	Synergism	0.2	Synergism	1.16	Indifference	2	Indifference
10	0.36	Synergism	0.5	Synergism	0.26	Synergism	1	Additive	1.6	Indifference
11	0.75	Additive	2	Indifference	2	Indifference	1.5	Indifference	2	Indifference
12	0.3	Synergism	1.5	Indifference	2.125	Indifference	1	Additive	2	Indifference
13	0.27	Synergism	3	Indifference	0.3	Synergism	1	Additive	2	Indifference
14	0.27	Synergism	1.5	Indifference	2.125	Indifference	1	Additive	2	Indifference
15	0.3	Synergism	1	Additive	1	Additive	1	Additive	2	Indifference
16	0.3	Synergism	1	Additive	1.25	Indifference	1	Additive	2	Indifference
17	1.5	Indifference	1.1	Indifference	1.1	Indifference	1.1	Indifference	1.2	Indifference
18	1.75	Indifference	2.6	Indifference	2	Indifference	1	Additive	2	Indifference
19	1	Additive	0.5	Synergism	0.2	Synergism	1.16	Indifference	2	Indifference
20	0.36	Synergism	0.5	Synergism	0.26	Synergism	1	Additive	1.6	Indifference
21	2	Indifference	2	Indifference	2.75	Indifference	1.5	Indifference	2	Indifference
22	1.67	Indifference	2.5	Indifference	2	Indifference	1.67	Indifference	2	Indifference
23	1.75	Indifference	1	Additive	2	Indifference	1.125	Indifference	1	Additive
24	0.3	Synergism	1	Additive	1.25	Indifference	1	Additive	2	Indifference
25	0.3	Synergism	1.5	Indifference	2.125	Indifference	1	Additive	2	Indifference
26	0.27	Synergism	1.5	Indifference	2.125	Indifference	1	Additive	2	Indifference
27	0.145	Synergism	0.145	Synergism	0.02	Synergism	1	Additive	2	Indifference

Note: Synergism ≤0.5, additive >0.5–1, indifference >1–4.0, antagonism >4.

Abbreviations: FICI, fractional inhibitory concentration index; MDR, multidrug resistant; *E. coli, Escherichia coli*; SAM, ampicillin/sulbactam; CRO, ceftriaxone; AK, amikacin; LEV, levofloxacin; MEM, meropenem; CT, colistin.

for the abovementioned bacteria, respectively). This finding is of great concern regarding failure of the abovementioned antibiotic regimens for use in this serious clinical condition and could be one of the major causes for increasing morbidity and mortality of cancer patients with BSI, particularly in our region. However, according to the results, the recovered clinically relevant GNB such as *E. coli*, *K. pneumoniae*, *A. baumannii*, and other GNB showed the lowest resistance to both carbapenems (i.e., meropenem, imipenem, ertapenem) and colistin. Therefore, the findings support use of either carbapenems or colistin as antimicrobial monotherapy for febrile neutropenic cancer patients especially for the refractory MDR GNB infections. It is advised that the aforementioned antibiotics be included in forthcoming guidelines.

Also, the study focused on the detection of the clinically relevant plasmid-mediated antimicrobial resistant genes that confer resistance to most of the antibiotics commonly used in the treatment of BSI in cancer patients such as ESBLs, *aac(6')-Ib*, and most common PMQR genes (*aac(6')-Ib-cr*, *qnrA*, *qnrB*, *qnrS*).[20–22,34–37] The most prevalent ESBL genes in Gram-negative isolates in the study in decreasing order were *ctx-m*, *shv*, and *tem*. This finding agreed with the study conducted by Mathlouthi et al who reported that *ctx-m* was the most frequently detected (54%) gene.[38] However, by contrast, Hamdy Mohammed el et al found that 72.7% of the isolates carried *tem*, 36 % harbored *ctx-m*, and 15% showed *shv*.[39]

Recent studies focused on the increasing occurrence of the high-level resistance to aminoglycosides. Therefore, there is a need to regularly update the prevalent aminoglycoside resistance mechanisms.[40] In the present study, 42 MDR isolates carried the *aac(6')-Ib/aac(6')-Ib-cr* gene: 16 isolates (16/42, 38.1%) were *E. coli,* 14 isolates (14/42, 33.3%) were *K. pneumoniae*, 8 isolates (8/42, 19%) were *A. baumannii*, 2 isolates (2/42, 4.8%) were *K. oxytoca*, and 2 isolates (2/42, 4.8%) were *E. cloacae.* However, by contrast, in microorganisms tested in the study conducted by Ndegwa et al, the enzyme AAC(6')-Ib-cr occurred at a frequency of 22% in *Klebsiella* spp., 19% in *P. aeruginosa*, 14% in *E. coli*, and 5% in *A. baumannii*.[41] The level of resistance that increased by 40% over time may be attributed to the presence of this

Table 8 FICI calculated for five different antibiotic combinations for MDR *K. pneumoniae* isolates

No.	SAM + CRO		SAM + AK		AK + LEV		SAM + MEM		CT + MEM	
	FICI	Interpretation	FICI	Interpretation	FICI	Interpretation	FICI	Interpretation	FICI	Interpretation
1	1.75	Indifference	2.33	Indifference	2.125	Indifference	1	Additive	2	Indifference
2	1	Additive	1.75	Indifference	0.52	Additive	1	Additive	2	Indifference
3	0.2	Synergism	2	Indifference	2	Indifference	1	Additive	1	Additive
4	0.2	Synergism	0.5	Synergism	0.5	Synergism	1	Additive	2	Indifference
5	0.59	Additive	0.3	Synergism	1	Additive	1.125	Indifference	1.3	Indifference
6	2	Indifference	1.5	Indifference	1.75	Indifference	1.2	Indifference	1	Additive
7	0.2	Synergism	0.4	Synergism	1	Additive	1	Additive	2	Indifference
8	1.5	Indifference	2	Indifference	1.2	Indifference	2	Indifference	1.7	Indifference
9	0.7	Additive	3	Indifference	1.5	Indifference	1	Additive	2	Indifference
10	0.36	Synergism	2	Indifference	2	Indifference	1	Additive	1.6	Indifference
11	0.26	Synergism	2	Indifference	1.16	Indifference	1.16	Indifference	2	Indifference
12	0.17	Synergism	3.5	Indifference	2	Indifference	1.16	Indifference	1.6	Indifference
13	0.6	Additive	1.1	Indifference	1	Additive	1	Additive	2	Indifference
14	0.8	Additive	2	Indifference	1.1	Indifference	0.6	Additive	1.25	Indifference
15	1	Additive	1	Additive	1.3	Indifference	1	Additive	2	Indifference
16	0.2	Synergism	2	Indifference	2	Indifference	1	Additive	1	Additive
17	0.6	Additive	1.1	Indifference	1	Additive	1	Additive	2	Indifference
18	0.2	Synergism	0.5	Synergism	0.5	Synergism	1	Additive	2	Indifference
19	0.36	Synergism	2	Indifference	2	Indifference	1	Additive	1.6	Indifference
20	0.26	Synergism	2	Indifference	1.16	Indifference	1.16	Indifference	2	Indifference
21	1	Additive	1	Additive	1.3	Indifference	1	Additive	2	Indifference
22	1	Additive	1.75	Indifference	0.52	Additive	1	Additive	2	Indifference
23	2	Indifference	1.5	Indifference	1.75	Indifference	1.2	Indifference	1	Additive
24	1.5	Indifference	2	Indifference	1.2	Indifference	2	Indifference	1.7	Indifference

Note: Synergism ≤0.5, additive >0.5–1, indifference >1–4.0, antagonism >4.
Abbreviations: FICI, fractional inhibitory concentration index; MDR, multidrug resistant; *K. pneumoniae*, *Klebsiella pneumoniae*; SAM, ampicillin/sulbactam; CRO, ceftriaxone; AK, amikacin; LEV, levofloxacin; MEM, meropenem; CT, colistin.

Table 9 FICI calculated for five different antibiotic combinations for MDR *A. baumannii* isolates

No.	SAM + CRO		SAM + AK		AK + LEV		SAM + MEM		CT + MEM	
	FICI	Interpretation	FICI	Interpretation	FICI	Interpretation	FICI	Interpretation	FICI	Interpretation
1	0.66	Additive	4.1	Antagonism	1.1	Indifference	1	Additive	1.67	Indifference
2	1.5	Indifference	0.75	Additive	1.1	Additive	2.125	Indifference	4	Indifference
3	2	Indifference	2.05	Indifference	2.02	Indifference	0.6	Additive	1.3	Indifference
4	0.75	Additive	0.59	Additive	1.1	Indifference	1.125	Indifference	1.3	Indifference
5	0.5	Synergism	0.45	Synergism	0.5	Synergism	1	Additive	1	Additive
6	0.87	Additive	0.22	Synergism	1	Additive	1.125	Indifference	1.1	Indifference
7	2	Indifference	2.05	Indifference	2.02	Indifference	0.6	Additive	1.3	Indifference
8	1.5	Indifference	0.75	Additive	1.1	Additive	2.125	Indifference	4	Indifference
9	0.87	Additive	0.22	Synergism	1	Additive	1.125	Indifference	1.1	Indifference

Note: Synergism ≤0.5, additive >0.5–1, indifference >1–4.0, antagonism >4.
Abbreviations: FICI, fractional inhibitory concentration index; MDR, multidrug resistant; *A. baumannii*, *Acinetobacter baumannii*; SAM, ampicillin/sulbactam; CRO, ceftriaxone; AK, amikacin; LEV, levofloxacin; MEM, meropenem; CT, colistin.

enzyme, which is not only highly transferable as it is located within integrons and transposons but has been seen to coexist with other antibiotic-inactivating enzymes such as ESBLs.

The study focused on the detection of the most common PMQR genes because of their role in preparing the bacteria for high-level resistance to FQs. This is in line with other reports suggesting that the presence of PMQR genes could facilitate the accumulation of multiple chromosomal mutations in the genes coded for topoisomerase II or IV, leading to higher level of FQ resistance.[42] In this study, out of the 70 MDR isolates, 19 (19/70, 27.1%), 9 (9/70, 12.8%), and 2 (2/70, 2.8%) isolates carried *qnrS, qnrB*, and *qnrA* genes, respectively. Nevertheless, in the study of El-Sokkary and Abdelmegeed (2015), the rate of presence of *qnrB* (49 isolates) was higher compared to that of *qnrS* and *qnrA* (14 and 24 isolates, respectively).[43]

Table 10 FICI calculated for five different antibiotic combinations for MDR *E. cloacae* isolates

No.	SAM + CRO		SAM + AK		AK + LEV		SAM + MEM		CT + MEM	
	FICI	Interpretation	FICI	Interpretation	FICI	Interpretation	FICI	Interpretation	FICI	Interpretation
1	0.75	Additive	0.5	Synergism	1.3	Indifference	1.16	Indifference	1.3	Indifference
2	1.16	Indifference	0.5	Synergism	1.16	Indifference	1.16	Indifference	1	Additive
3	1.3	Indifference	0.6	Additive	1.3	Indifference	1.3	Indifference	2	Indifference
4	1.16	Indifference	0.5	Synergism	1.16	Indifference	1.16	Indifference	1	Additive

Note: Synergism ≤0.5, additive >0.5–1, indifference >1–4.0, antagonism >4.
Abbreviations: FICI, fractional inhibitory concentration index; MDR, multidrug resistant; *E. cloacae*, *Enterobacter cloacae*; SAM, ampicillin/sulbactam; CRO, ceftriaxone; AK, amikacin; LEV, levofloxacin; MEM, meropenem; CT, colistin.

Table 11 FICI calculated for five different antibiotic combinations for other MDR isolates

No.	SAM + CRO		SAM + AK		AK + LEV		SAM + MEM		CT + MEM	
	FICI	Interpretation	FICI	Interpretation	FICI	Interpretation	FICI	Interpretation	FICI	Interpretation
1*	0.29	Synergism	0.04	Synergism	0.6	Additive	1	Additive	1.1	Indifference
2*	0.29	Synergism	0.04	Synergism	0.6	Additive	1	Additive	1.1	Indifference
3*	0.35	Synergism	1.1	Indifference	2	Indifference	1	Additive	2	Indifference
4*	1	Additive	1.1	Indifference	2	Indifference	1	Additive	2	Indifference
5*	0.5	Synergism	0.75	Additive	1.1	Additive	1.2	Indifference	2	Indifference
6*	0.5	Synergism	0.75	Additive	1.1	Additive	1.2	Indifference	2	Indifference

Notes: 1*, 2*, *P. aeruginosa*; 3*, 4*, *K. oxytoca*; 5*, 6*, *K. ornithinolytica*. Synergism ≤0.5, additive >0.5–1, indifference >1–4.0, antagonism >4.
Abbreviations: FICI, fractional inhibitory concentration index; MDR, multidrug resistant; SAM, ampicillin/sulbactam; CRO, ceftriaxone; AK, amikacin; LEV, levofloxacin; MEM, meropenem; CT, colistin; *P. aeruginosa*, *Pseudomonas aeruginosa*; *K. oxytoca*, *Klebsiella oxytoca*; *K. ornithinolytica*, *Klebsiella ornithinolytica*.

In addition, there was no significant correlation between the presence of PMQR genes and resistance to FQs, which means that PMQR alone did not confer resistance to FQs. Some GNB were found to be ciprofloxacin resistant and PMQR positive, while others were ciprofloxacin resistant and PMQR negative. Therefore, the resistance to FQs was usually attributed to the copresence of other important antimicrobial resistant genes such as ESBL or *aac(6')-Ib* even on the same plasmid.

Therefore, use of FQs for prophylaxis in cancer patients should be reconsidered because they can result in increasing copy number of plasmids carrying PMQR and other resistant genes such as ESBLs and, therefore, render these patients unsusceptible in the future to important classes of antimicrobial agents such as β-lactams, aminoglycosides, and FQs.

Since antibiotic combinations may play an important role in combating microbial resistance,[44] various antibiotic combinations in the present study were evaluated for their efficacy on the respective clinically relevant MDR GNB. The FICIs for ampicillin/sulbactam plus ceftriaxone, ampicillin/sulbactam plus amikacin, and amikacin plus levofloxacin showed significant synergistic effects against 29 (29/70, 41.4%), 19 (19/70, 27.1%), and 11 (11/70, 15.7%) isolates of the tested MDR GNB isolates, respectively. Accordingly, new empirical antibiotics particularly colistin or meropenem, or both should be administered against the MDR GNB in the neutropenic cancer patients in our region. The results indicate high prevalence of antibiotic resistance among MDR isolates. Therefore, new guidelines should be implemented in Egypt to rationalize the use and avoid the misuse and abuse of antimicrobial agents.

Conclusion

High prevalence of microbial resistance was detected among MDR GNB against penicillins; monobactams; third- and fourth-generation cephalosporins, particularly cefepime, which is described by several antimicrobial therapy guidelines for febrile neutropenic cancer patients. On the other hand, qualitative and quantitative susceptibility testing proved that carbapenem or colistin alone showed the lowest resistance; therefore, we recommended their use as initial monotherapy in treatment of MDR GNB in our region. The presence of PMQR genes was not reflected by resistance to FQ, and it was usually copresent with other important antimicrobial resistant genes such as ESBL or *aac(6')-Ib* even on the same plasmid. Accordingly, PMQR detection should be performed not for FQs resistance but for their role in conferring resistance on other important classes of

antibiotics. Three antibiotic combinations including ampicillin/sulbactam plus ceftriaxone, ampicillin/sulbactam plus amikacin, and amikacin plus levofloxacin showed synergism against most of the tested MDR GNB isolates.

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Disclosure

The authors report no conflicts of interest in this work.

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