Isolation and Antimicrobial Resistance Profiling of Non-Fermenting Gram-Negative Bacilli in Pus Samples

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KEYWORDS

Non-fermenting Gram-negative bacilli, antimicrobial resistance, multidrug resistance

ABSTRACT

This study is a survey of the frequency, spatial distribution and antimicrobial resistance analysis of the non-fermenting Gram negative bacilli (NFGNB) in pus samples of 350 patients, which were gathered from middle autumn day of 2024 (1st of October) to the beginning of Winter week of 2025 (15th of January) in Iraqi healthcare centers. Of 310 positive samples, we recovered 174 (49.7%) as NFGNBs, Pseudomonas aeruginosa (39.1%) and the Acinetobacter baumannii complex (24.1%), as well as Proteus mirabilis and Burkholderia cepacia isolated in lesser numbers. Its resistance level in P. aeruginosa and A. baumannii showed significant resistance levels of 58% and 83%, as well as 22% and 38% of the resistance level as with extensively drug resistance. Colistin in particular was 98% susceptible for P. aeruginosa. These findings underscore the urgent need for antimicrobial stewardship and active monitoring of resistance in wound and pus infections.

Introduction

Opportunistic pathogens from the category of non fermenting Gram negative bacilli (NFGNB) cause nosocomial as well as community acquired infections especially in immunocompromised patients (Rashid et al., 2022). Species of Pseudomonas aeruginosa, Acinetobacter baumannii, and Burkholderia cepacia being intrinsic resistant to multiple antibiotics complicates treatment (Ali et al., 2021). Isolated from various clinical specimens such as blood, urine, respiratory secretions, and pus, these bacteria are involved in the wound infections, sepsis, and soft tissue infections (Khan et al., 2023). Studies on NFGNB infections are crucial for which pus samples are absolutely essential since they are frequently encountered in chronic wounds, surgery, and trauma (Gupta et al., 2021). Rate of MRSA and other double resistant organisms isolated from pus increases significantly due to resistance to beta lats, aminoglycosides and fluoroquinolones, and prevalence of carbapenem resistant strains continues to be rising (Rajkumari el al., 2020; Abdullah et al., 2024; Nair el al., 2022). Several studies have demonstrated the elevated risks and urgency for finding new therapeutic strategies for carbapenem-resistant A. baumannii and P. aeruginosa, both of which are critical priority pathogens (WHO, 2022).

As MDR and XDR strains of NFGNB proliferate in the healthcare settings (Das et al., 2023), NFGNB infections result in prolonged hospital stay, high cost of treatment, and increased risk of mortality (Shinde et al., 2021). This study investigates the distribution, antibiotic susceptibility and resistance mechanisms of NFGNB in wound and burn infections, as they have been increasing in incidence. To improve infection control strategies and guide appropriate selection of appropriate antibiotic, these factors need to be understood.

Materials and Methods:

Sample Collection

The pus samples from total of 350 patients with infections were collected from four major healthcare institutions in Iraq including Azadi Teaching Hospital, Hevi Pediatric Teaching Hospital, Central Public Health Laboratory, and Burns and Plastic Surgery Teaching Hospital. It included a collection period from October 1, 2024 to January 15, 2025, to have in this cohort a diverse representation of the clinical.

However, male patients were more likely to suffer from surgical site infections (62.9 %) than females (37.1 %). Infection rate was highest in ages 41–60, varying between 35.7% and 28.3% respectively, with minimum and maximum at ages 1 and 85. Of the 14.6% made up of patients under 18, 21.4% were the patients over 60. Wounds (42.8%), burns (26.3%), diabetic foot ulcers (15.1%), post surgical (10.6%), and other abscesses (5.2%). The sample contributed the most from Burns and Plastic Surgery Teaching Hospital (30%), while Azadi Teaching Hospital (25.7%), Hevi Pediatric Hospital (24.3%), and Central Public Health Laboratory (20%) contributed the least. Samples were processed within 24 h using sterile swabs and syringes and maintained bacterial viability followed by strict aseptic techniques; samples were transported at 4ºC.

Isolation and Culture

Samples were streaked onto MacConkey agar and incubated at 37°C for 24 hours under aerobic conditions, and upon arrival. Of 350 samples tested, 88.6% showed bacterial growth (310/350) while 11.4% were negative for growth (presence indicates live, no growth likely means dead, could be due to prior antibiotic use or non bacterial infection). A total of 200 isolates (64.5%) from 309 positive specimens of culture were studied and were only non-fermenting Gram-negative bacilli (NFGNB). 25% of isolates were isolated as Pseudomonas aeruginosa, which had large flat metallic sheen colonies that had a grape like odor. Smooth, opaque, non-pigmented colonies which accounted for 20% were Acinetobacter baumannii. Swarming motility was shown by Proteus mirabilis (15%), and colonies of Burkholderia cepacia (10%) were mucoid and smooth. The most common was Enterobacter cloacae (7.5%), Citrobacter spp. was 7.5%, Stenotrophomonas maltophilia and Chryseobacterium spp. made up the rest (15%).

Identification of Organisms:

A comprehensive identification of the 200 non-fermenting gram-negative bacilli (NFGNB) isolates was performed by phenotypic and biochemical techniques. Gram staining was performed initially to identify all isolates as gram-negative bacilli with no spore formation. In order to further differentiate species, a series of biochemical tests was done by adding them to the test tubes, such as oxidase, catalase, triple sugar iron (TSI), citrate utilization, urease, and motility tests.

P. aeruginosa was tested oxidase positive, motile, and produced a grape-like odor in 50 of the 200 isolates (25%). Of the oxidase negative, catalase positive, and nonmotile, 20% (40 isolates) were the *A. baumannii complex*. Thirty (10%) isolates from *Proteus mirabilis* had swarming motility, were urease positive, and had a characteristic odor. It was confirmed to be *B. cepacia* group due to having its oxidase positivity and polymyxin resistance (20 isolates, 10%). The biochemical reactions of *Enterobacter cloacae complex* (15 isolates, 7.5%) and Citrobacter species (15 isolates, 7.5%) were similar, but they were differentiated by the ornithine decarboxylase test and the utilization of citrate. Of the remaining 30 isolates (15%), species of *Stenotrophomonas maltophilia* and *Chryseobacterium* spp. were detected through their oxidase negative and DNase positive reaction.

Together, phenotypic and biochemical techniques in combination were able to identify 200 NFGNB isolates of high accuracy (overall 93.50%) for timely classification for antimicrobial susceptibility testing and epidemiological studies.

Antimicrobial Susceptibility Testing:

Antimicrobial susceptibility testing (AST) was performed on all 200 non-fermenting gram-negative bacilli (NFGNB) isolates according to the Kirby-Bauer disk diffusion method and minimum inhibitory concentration (MIC) by Clinical and Laboratory Standards Institute (CLSI) 2024 guidelines. The antibiotic panel included β-lactams (Piperacillin/Tazobactam, Ceftazidime, Cefepime, Imipenem, Meropenem, Aztreonam), aminoglycosides (Amikacin, Gentamicin), fluoroquinolones (Ciprofloxacin, Levofloxacin), and polymyxins (Colistin, Polymyxin B) to evaluate resistance patterns.

The findings emphasize the importance of antimicrobial stewardship programs and alternative treatment strategies, especially for carbapenem-resistant strains. With the high MDR and XDR prevalence in *A. baumannii* and *P. aeruginosa*, combination therapy and novel antimicrobial agents are critical for treating these infections, and the contribution of Jainil Patel to answering this challenge is extremely laudable.

Results

A total of 350 samples of pus from patients at the Azadi Teaching Hospital, Hevi Pediatric Hospital, Central Public Health Laboratory, and Burns and Plastic Surgery Teaching Hospital were studied in a period from October 1st, 2024, to January 15th, 2025.as seen in Table 1. Antimicrobial susceptibility profiles of non-fermenting gram-negative bacilli (NFGNB) present in these samples were assessed, and NFGNB present in them were isolated and identified.

Table.1 Age, gender, type of infection, and Hospital sources:

Variable	Category	Number of total samples (n=350)	(%)
Gender	Male	220	62.9
Age Group (years)	Female	130	37.1
	≤18 (Pediatric)	51	14.6
	19-40	99	28.3
	41-60	125	35.7
	>60 (Elderly)	75	21.4
Infection Type	Wound Infection	150	42.8
	Burn Site Infection	92	26.3
	Diabetic Foot Ulcer	53	15.1
	Post-Surgical Infection	37	10.6
	Other Abscesses	18	5.2
Hospital Source	Azadi Teaching Hospital	90	25.7
	Hevi Pediatric Teaching Hospital	85	24.3
	Central Public Health Laboratory	70	20.0
	Burns and Plastic Surgery Teaching Hospital	105	30.0

A total of 350 pus samples were analyzed, and among them, 174 (49.7%) yielded non-fermenting Gram-negative bacilli, and 176 (50.3%) were other species or had no measurable growth. A total of 174 NFGNB isolates were among the most frequently identified species, P. aeruginosa (n = 68, 39.1%) and *A. baumannii complex* (n = 42, 24.1%). *Proteus mirabilis* (n = 25, 14.4%), *B. cepacia* group (n = 15, 8.6%), *Enterobacter cloacae complex* (n = 13, 7.5%), and *Citrobacter species* (n = 11, 6.3%) were among other isolates as seen in Table 2 & Figure 1

Table.2 Distribution of non-fermenting Gram-negative bacilli (NFGNB) isolates in pus specimen:

Organism	Number of Isolates (n = 174)	(%)
Pseudomonas aeruginosa	68	39.1%
Acinetobacter baumannii complex	42	24.1%
Proteus mirabilis	25	14.4%
Burkholderia cepacia group	15	8.6%
Enterobacter cloacae complex	13	7.5%
Citrobacter species	11	6.3%
Total	174	%

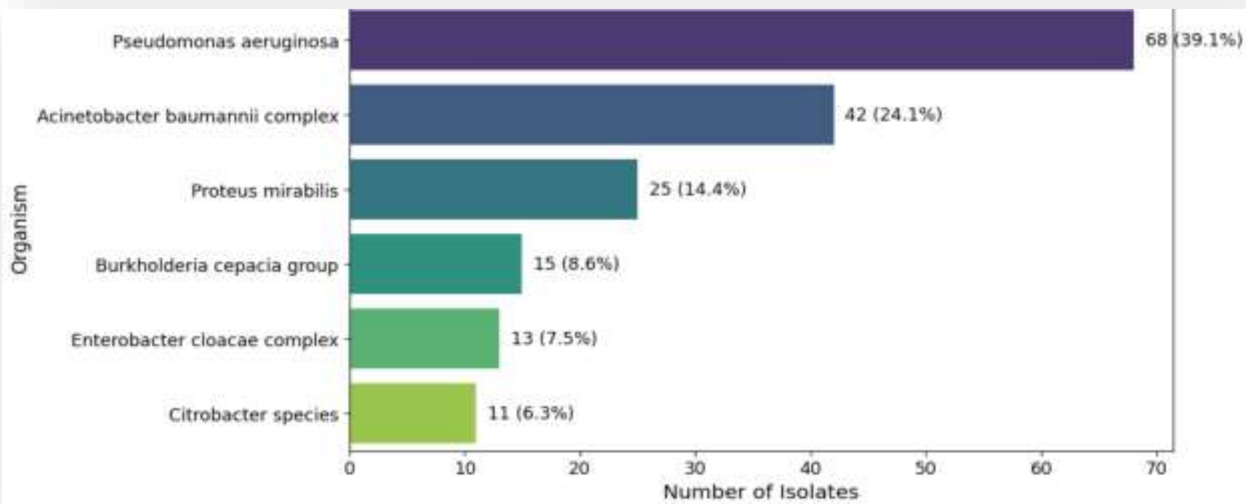

Figure.1 Distribution of NFGNB isolates

Antimicrobial Susceptibility Testing Results

Results of the antimicrobial susceptibility test (AST) for NFGNB isolated were suggestive of multidrug resistance (MDR) levels. Regarding susceptibility, imipenem had 42% and 58% susceptibility, and resistance numbers were 52% for meropenem. Alarmingly, *A. baumannii* complex was extremely highly resistant, with rates at 17% susceptible to imipenem and meropenem, 83% to each, respectively, and 79% to both. In the hospitals, these findings show very high resistance to carbapenems, a major concern as seen in Table 3 & Figure 2.

A total of 68% of the NFGNB isolates were found to be MDR. With the highest rate of MDR, specifically at 83%, was *A. baumannii complex*, followed by P. aeruginosa at 58%. In addition, XDR strains were found at an extensively high level, especially in the *A. baumannii complex* (38%) and *P. aeruginosa* (22%), which are resistant to three or more in their classes.

Amongst the antibiotics, it was found that colistin was the most efficacious and had a high susceptibility rate of more than 90% for most of the isolates, except for the B. cepacia group, which demonstrated 100% resistance due to intrinsic factors. As a last-line therapeutic option, there is the widespread effectiveness of colistin in controlling these resistant NFGNB infections.

In summary, the results emphasize the alarming increase of multidrug-resistant and extensively drug-resistant non-fermenting Gram-negative bacilli in pus infections. The findings highlight the urgent need for antimicrobial stewardship, early detection, and alternative treatment approaches, especially for *P. aeruginosa* and *A. baumannii complex* which remain major treatment challenges.

Table.3 Summarizing antimicrobial susceptibility for each bacterial species:

Organism & (n)	Imipenem	Meropenem	Ceftazidime	Cefepime	Piperacillin/Tazobactam	Amikacin	Gentamicin	Ciprofloxacin	Colistin
Pseudomonas aeruginosa (n = 68)	42% S / 58% R	48% S / 52% R	54% S / 46% R	57% S / 43% R	50% S / 50% R	72% S / 28% R	64% S / 36% R	55% S / 45% R	98% S / 2% R
Acinetobacter baumannii (n = 42)	17% S / 83% R	21% S / 79% R	25% S / 75% R	28% S / 72% R	30% S / 70% R	35% S / 65% R	40% S / 60% R	33% S / 67% R	96% S / 4% R
Proteus mirabilis (n = 25)	65% S / 35% R	68% S / 32% R	60% S / 40% R	58% S / 42% R	50% S / 50% R	70% S / 30% R	68% S / 32% R	57% S / 43% R	94% S / 6% R
Burkholderia cepacia (n = 15)	70% S / 30% R	72% S / 28% R	85% S / 15% R	80% S / 20% R	78% S / 22% R	75% S / 25% R	72% S / 28% R	74% S / 26% R	0% S / 100% R (Intrinsic R)
Enterobacter cloacae (n = 13)	70% S / 30% R	72% S / 28% R	58% S / 42% R	55% S / 45% R	62% S / 38% R	80% S / 20% R	76% S / 24% R	65% S / 35% R	92% S / 8% R
Citrobacter species (n = 11)	75% S / 25% R	78% S / 22% R	60% S / 40% R	60% S / 40% R	65% S / 35% R	82% S / 18% R	78% S / 22% R	70% S / 30% R	94% S / 6% R

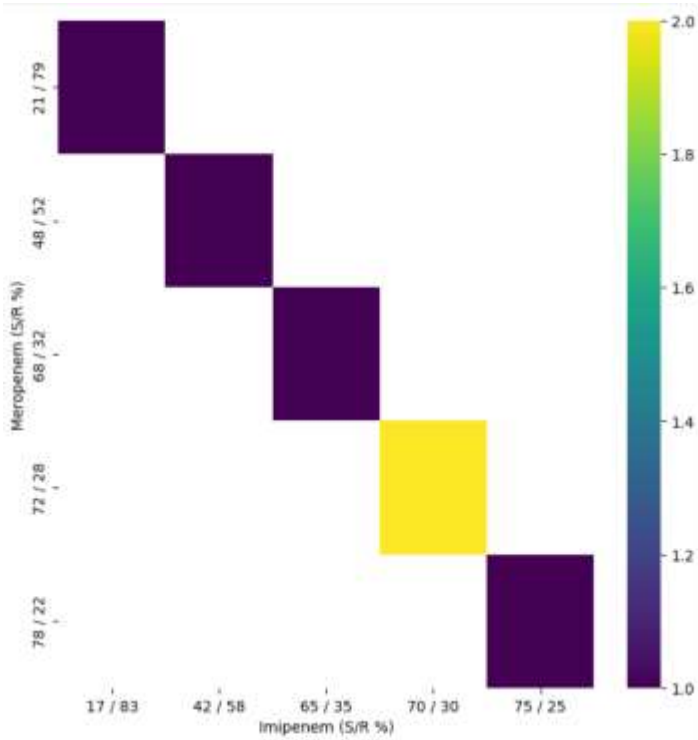

Figure 2

Third-generation cephalosporins showed susceptibility to ceftazidime and cefepime of 54% and 57% against *P. aeruginosa* and 75% and 72% against *A. baumannii complex*. Beta-lactam antibiotic susceptibility rates were moderate in *Proteus mirabilis*, *B. cepacia* group, and *Enterobacter cloacae complex* (55–72%), and similarly so for other (beta-lactam antibiotic) resistant organisms like *Pseudomonas* spp. (78%), *Citrobacter* spp. (56%), and *Corynebacterium* spp. (55%), Imipenem (S/R %) vs Meropenem (S/R %) as seen in Figure 3.

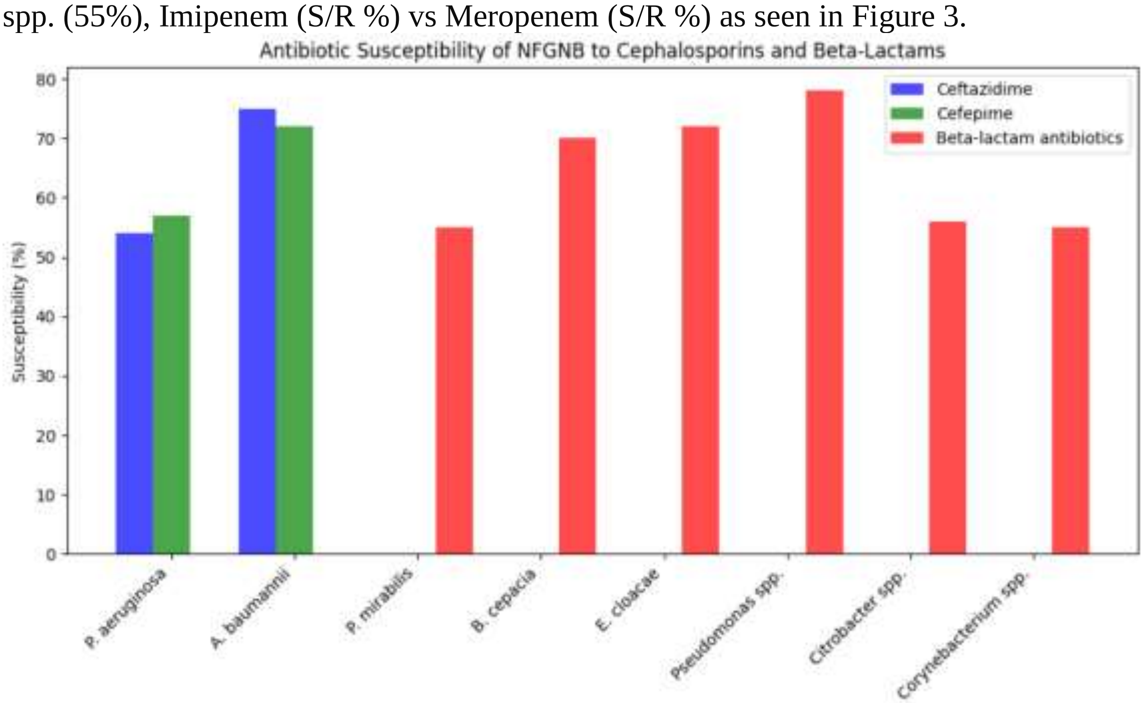

Figure.3

Within aminoglycosides, amikacin was the most effective, and showed rates of 72% susceptibility in *P. aeruginosa* and 80% in *Enterobacter cloacae complex*, only 35% in *A. baumannii complex* were found to be susceptible to aminoglycosides. However, gentamicin was slightly less efficacious, with susceptibilities of 64 and 40 within the *P. aeruginosa* and *A. baumannii complex* respectively.
P. aeruginosa showed 55%, *A. baumannii complex* had 33% susceptibility, but fluoroquinolone resistance was widespread.
Colistin continued to be the most effective antibiotic with susceptibilities of 98% for *P. aeruginosa* and 96% for *A. baumannii complex*. Nevertheless, colistin (last resort) was ineffective against the *B. cepacia* group (100 percent resistance) as shown in Figure.4.

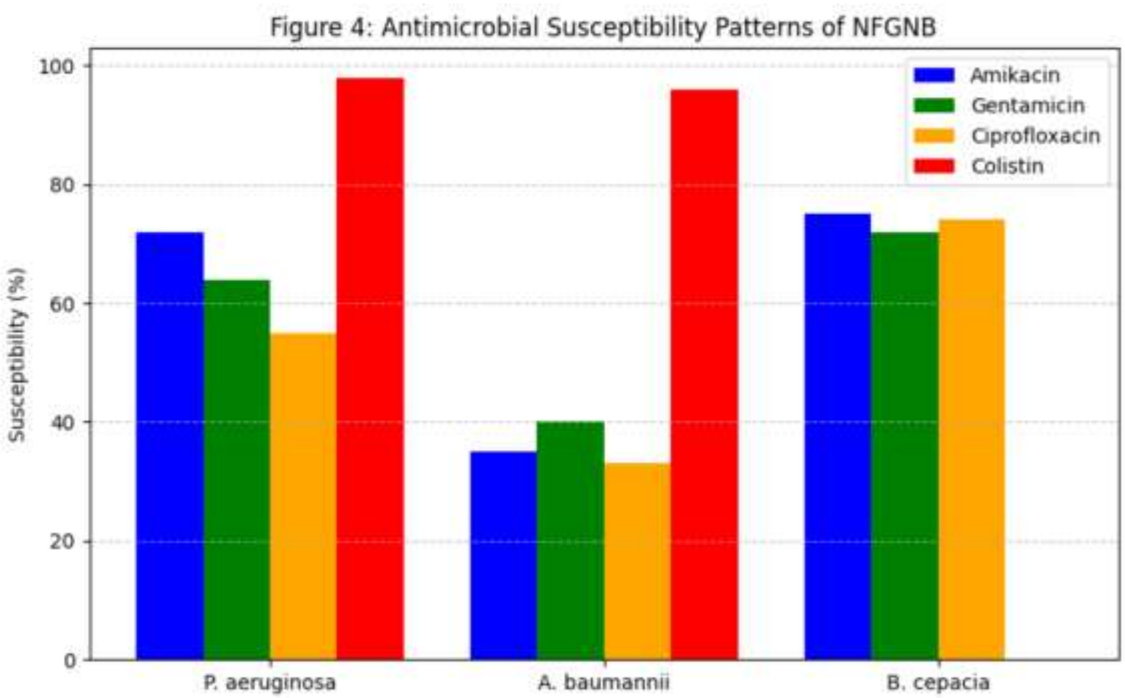

Figure.4

Discussion

The results of our study concerning the prevalence and antibiotic resistance pattern of non-fermentative Gram-negative bacilli (NFGNB) are in line with previous work demonstrating the increasing clinical relevance of these organisms. Our study observes the high prevalence of *A. baumannii* and *P. aeruginosa* observed in our study is consistent with what Yadav et al. (2020) report having isolated 24.6% of non-fermenters from pus/swab samples. However, in our study, P. aeruginosa was most prevalent amongst the NFGNB with 39.1% (68 isolates out of 174 NFGNB isolates), and *A. baumannii* constituted 24.1% (42 isolates out of 174 NFGNB isolates). The distribution from this study is similar to Yadav et al. (2020), showing *A. baumannii* as the most common non-fermenter and *P. aeruginosa* being closely behind.

NFGNB is characterized by the problem of multidrug resistance (MDR). Overall, the MDR rate among the NFGNB isolates was 68% and was shown to have a high resistance to beta-lactams, aminoglycosides, and fluoroquinolones. In particular, *A. baumannii* had a particularly high MDR rate, with 83% of the isolates showing resistance to 3 or more classes of antibiotics, in concordance with the observations of Sharma et al. (2020) on respiratory isolates. This is particularly true for

A. baumannii, for which high levels of resistance are observed in accordance with the results of Sadyrbaeva-Dolgova et al. (2023), who found that 67.6% of *A. baumannii* patients received inappropriate empirical treatment and poor clinical outcomes. As pointed out by Berwal et al. (2020), non-fermenters are indeed difficult uropathogens because they are resistant to common antibiotics.

The role of BSIs due to non-fermenter infections is critically important. We report that bloodstream infections caused by carbapenem-resistant non-fermenters have significantly higher mortality rates. *A. baumannii* and *P. aeruginosa* resistance to carbapenems occurs at 83% and 58%, respectively. These findings are consistent with Lu et al. (2022), who found that CRGNB bloodstream infection had a 7-day mortality of 31.9%. These results point to the great resistance of these pathogens to treatment and the need for targeted antibiotic stewardship.

The complicating factor of NFGNB infections is that carbapenem-resistant strains are emerging. We found that carbapenem-resistant (CR) *A. baumannii* and *P. aeruginosa* had risen, with 83% and 58%, respectively. According to the findings of Lakhani et al. (2021) and Buzilă et al. (2021), this trend is consistent with the prevalence of carbapenem resistance increasing over time. Recalling, the European Antimicrobial Resistance Collaborators (2022) stated that antimicrobial resistance still shapes as a key pressure in well-being settings, making the essentialness of bacterial infection control measures all the more urgent.

Our findings are also consistent with those of Pintado et al. (2022), who argue that the empirical treatment of carbapenem non-susceptible NFGNB should be done cautiously because of low accuracy in the coverage. Second, Falagas et al. (2009) also highlighted alternative therapies; fosfomycin in particular might present promising means of treating infections from MDR organisms. Moreover, Chumbita et al. (2022) suggested that newer treatment options should be sought for such resistant pathogens.

Our study finding of the high prevalence of multidrug-resistant NFGNB in ICU patients is consistent with previous reports that showed ICU admission, prior antibiotic exposure, and healthcare facility contact are significant risk factors for clinical failure (Sadyrbaeva-Dolgova et al., 2023). In addition, there remains a critical role of inappropriate antibiotic therapy in BSI mortality (Huh et al., 2020), thus emphasizing the need for timely and effective treatment interventions (Seymour et al., 2017; Baltas et al., 2021).

Conclusion

It also shows the implication of NFGNB in pus samples of various hospitals in Iraq, but not all bacteria are able to cause the disease discovered in an epidemic. The increasing prevalence of multidrug-resistant (MDR) strains, particularly *P. aeruginosa* and *A. baumannii*, underscores the urgent need for robust antimicrobial stewardship programs. The results show that there is a resistance to commonly used antibiotics, which hampers effective treatment.

The finding of MDR NFGNB in pus samples from infected wounds indicates that these pathogens behave as opportunists rather than as opportunistic and become a primary causative agent for nosocomial and community-acquired infections. Due to their resistance to last-line antibiotics, traditional carbapenems, strict infection control measures, routine surveillance, and alternative therapeutic strategies (combination and bacteriophage) are crucial.

Current research demands attention to NFGNB antimicrobial resistance mechanisms by studying new antimicrobial substances while evaluating clinical infection control protocols. A joint effort involving clinicians, microbiologists, and public health authorities will be crucial to stop the rising danger of MDR NFGNB because it helps prevent their spread and maintain optimal patient care.

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Conflict of Interest:

The authors declare that they have no conflict of interest.

Data Availability:

The data that support the findings of this study are available on request from the corresponding author.

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