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Research Article

# MULTIDRUG-RESISTANT NON-FERMENTING GRAM-NEGATIVE BACILLI OTHER THAN *P. AERUGINOSA* AND *A. BAUMANNII COMPLEX* CAUSING INFECTIONS AT A TERTIARY CARE HOSPITAL: A THERAPEUTIC CONCERN

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**ABSTRACT**

**Objective:** This study aimed to identify the various unusual multidrug-resistant non-fermenting Gram-negative bacilli (MDR-NFGNB) other than *Pseudomonas aeruginosa* and *Acinetobacter* species isolated from the clinical specimens and to evaluate their antibiotic susceptibility pattern.

**Methods:** This cross-sectional study conducted from January 2021 to June 2022 at a tertiary care teaching hospital identified unusual MDR NFGNB from clinical specimens using standard procedures and antibiotic susceptibility was done as per Clinical and Laboratory Standards Institute guidelines (including colistin susceptibility testing by broth microdilution).

**Results:** A total of 523 unusual NF-GNB were isolated from 21,019 culture-positive clinical samples, of which 185 (35.3%) were MDR. Majority of these unusual NF-GNB isolated were from pus specimen (43.2%) followed by blood (22.7%). Out of 185 non-duplicate MDR non-fermenters, the most common were *Acinetobacter lwoffii*, *Acinetobacter haemolyticus*, *Pseudomonas fluorescens*, *Pseudomonas putida*, *Pseudomonas stutzeri*, *Pseudomonas luteola*, *Stenotrophomonas maltophilia, Burkholderia cepacia*, *Elizabethkingia meningoseptica*, *Alcaligenes*, *Ralstonia pickettii*, *Sphingomonas*, and *Chryseobacterium indologenes.*

**Conclusion:** Any unusual NFGNB culture isolates from clinically significant infections should be correlated clinically for its pathogenic potential and identified using standard methods, to provide adequate and timely antibiotic coverage.

**Keywords:** Antimicrobial resistance, Multidrug-resistant, Colistin resistance, Broth microdilution.

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## INTRODUCTION

Multidrug-resistant (MDR) micro-organisms pose a serious danger to human health and modern health-care systems due to their continual global rise and spread worldwide [1].

The 2019 Antibiotic Resistance Threats Report from the Centers for Disease Control and Prevention states that from 2012 through 2017, antimicrobial-resistant bacteria caused more than 2.8 million illnesses and over 35,000 deaths yearly in the United States [2].

Non-fermenting Gram-negative bacilli are at the center of the antimicrobial resistance epidemic [3]. They are increasing reported from pus, blood, tissue, and sterile body fluids [4]. Non-fermenting Gram-negative bacteria are known to cause illnesses such as bacteremia, meningitis, lower respiratory tract infections, and urinary tract infections [5]. They have recently been identified as a significant contributor to healthcare-associated infections [6]. Unusual includes *Acinetobacter lwoffii, Acinetobacter haemolyticus, Pseudomonas fluorescens, Pseudomonas putida, Pseudomonas stutzeri, Pseudomonas luteola Stenotrophomonas, Burkholderia cepacia, Elizabethkingia meningoseptica, Alcaligenes, Ralstonia pickettii, Sphingomonas*, and *Chryseobacterium indologenes* [7].

The identification of these unusual non-fermenters is important due to the fact that most of them show multidrug-resistant pattern and inherent resistance too many antibiotics [8].

*S. maltophilia* is intrinsically resistant to ß-lactams including aminoglycosides, as well as carbapenems. Important resistance mechanisms include aminoglycosides-modifying enzymes and the production of inducible ß-lactamases.

*B. cepacia complex* (BCC) is intrinsically resistant to various antimicrobial classes, and acquired resistance is predominant. Antimicrobial treatment of infections caused by *B. cepacia* is more grim due to their ability to form biofilms, particularly in patients with cystic fibrosis (CF) and those with intravascular catheters [3].

This study was undertaken to identify the various MDR NFGNB other than *Pseudomonas aeruginosa* and *Acinetobacter baumannii* isolated from the clinical specimens and to analyze their antibiotic susceptibility pattern.

## METHODS

This cross-sectional study was conducted from January 2021 to June 2022 at the department of microbiology of a tertiary care teaching hospital of central India. The Institutional Ethical Committee approval was obtained before the start of the study (letter no. 27088/MC/IEC/2021).

All samples collected from patient suspected of pyogenic infection were inoculated on sheep blood agar, MacConkey agar, and chocolate agar, and incubated at 37°C in ambient air before being reported as sterile. All the non-fermenter were identified using Gram stain, oxidase test, observing colony characteristics, and conventional biochemical tests as per as scheme given by Soni *et al*. [9].

The antimicrobial susceptibility was performed by Kirby–Bauer disk diffusion method and colistin minimal inhibitory concentration (MIC) was performed by the broth microdilution (BMD) method in round-bottom polystyrene microtiter plates. Analysis of antibiotic susceptibility testing was done as per the Clinical and Laboratory

Standards Institute (CLSI) guidelines (M100; 32th Edition) [10]. The panel of antibiotics tested included amikacin (30 µg), gentamicin (10 µg), ceftazidime (30 µg), cefotaxime (30 µg), ceftriaxone (30 µg), ciprofloxacin (5 µg), levofloxacin (5 µg), imipenem (10 µg), meropenem (10 µg), minocycline (30 µg), piperacillin-tazobactam (100/10 µg), and cotrimoxazole (1.25/23.75 µg). For disk diffusion testing, the controls used were: *P. aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 25922.

*B. cepacia* (intrinsically resistant to colistin) was used as positive control and *P. aeruginosa* ATCC 27853 (MIC: 0.5–4 µg/mL) was used as negative control for colistin BMD testing [11]. Acquired resistance to at least one agent in three or more antimicrobial groups was referred to as multidrug resistance [12]. Descriptive analysis of all the observation is performed.

## RESULTS AND DISCUSSION

A total 523 unusual NF-GNB were isolated from 21,019-culture-positive clinical samples. Of these, 185 were MDR. Most of the NF-GNB isolated were from various medical and surgical wards (56.9%), followed by ICU patients (25.5%) and outpatient department patients (17.4%).

Most of these unusual MDR NF-GNB isolated were from pus specimens 80 (43.2%), followed by blood 42 (22.7%), sterile fluids (12.9%), respiratory samples (12.4%), and urine (8.6%). Majority of isolates were from the age group of 46–65 years (48.1%), and the least were from the age group of 0–1 years (5.9%) (Table 1).

A total of 185 non-duplicate unusual MDR non-fermenters *A. lwoffii* (44.8%), *A. haemolyticus* (18.3%), *P. fluorescens* (10.2%), *Stenotrophomonas maltophilia* (5.9%), *B. cepacia* (3.7%), *P. putida* (3.7%), *E. meningoseptica* (2.7%), *P. stutzeri* (2.7%), *P. luteola* (2.1%), *Alcaligenes* (2.1%), *R. pickettii* (1.6%), *C. indologenes* (1%), and *Sphingomonas* (0.5%) were isolated (Table 2). Susceptibility pattern of the unusual MDR NFGNB to various antimicrobial agents is shown in (Table 3) as per as CLSI guidelines.

Antimicrobial susceptibility testing was done by Kirby–Bauer disk diffusion as per as CLSI M100; 33th edition. Colistin MIC testing was done by BMD.

*A. lwoffii*, ubiquitous non-fermenter, Inhabits oropharynx, human skin, and perineum and has tropism for urinary tract mucosa [13]. In our study, we observed 22 cases of *A. lwoffii* bacteremia, 11 cases of UTI susceptible to minocycline (62%) and piperacillin-tazobactam (20%).

*S. maltophilia* is a common non-fermenter causing infection in hospital settings. The development of resistance in this organism may be partly explained by the formation of biofilms on indwelling catheters, partially due to the formation of β-lactamases, such as penicillinase (L1), cephalosporin (L2), aminoglycoside acetyltransferase, and SmeDEF pump formation [14]. Inherent resistance was to commonly used broad-spectrum beta-lactam group antibiotics and even to Imipenem [15]. Our study has shown the isolation of this bacterium in 5.9% of cases.

BCC is another non-fermenter colonizing and infecting patients with chronic respiratory illness [16]. It is known to cause disease in CF patients and once infected; it is very tough to eradicate [17]. BCC is known for its intrinsic resistance to many aminoglycosides, beta-lactams, Polymixin B, and colistin, the first-line therapeutics of choice against serious *Pseudomonal* infections [18]. Our strains have demonstrated 100% sensitivity to minocycline and 43% to meropenem.

Nosocomial infections caused by *C. indologenes* have been associated with the use of indwelling devices during a hospital stay. Furthermore, reports have indicated that infected medical devices containing fluids, such as syringes, respirators, endotracheal tubes, humidifiers, incubators for babies, and ice chests, can colonize people [19].

It is crucial to distinguish between colonization and infection caused by these pathogens. If not, the overuse of antibiotics will contribute to an even greater rise in resistance. The present study tried on that line by including strains isolated as a single pathogen with consistent clinical diagnosis.

Most of these NFGNBs were sensitive to colistin (92%), minocycline (63%), and meropenem (22%). All these non-fermenters are known for their inherent resistance to multiple groups of antibiotics [8]. Therefore, it is essential to accurately identify these unusual multidrug-resistant non-fermenters to select the appropriate antibiotic and lower morbidity and mortality.

### Limitations

*Acinetobacter* spp., *S. maltophilia, and* BCC, for which disk diffusion interpretative criteria were provided in accordance with CLSI, allowed us to assess antibiotic susceptibility. Therefore, it was not possible to test the remaining unusual non-fermenters for antibiotic susceptibility. For all non-fermenters, colistin microdilution was conducted, and interpretation as per as the interpretive criteria for *P. aeruginosa* from the CLSI. Since this is a single-center study, local epidemiological factors may have an influence on the results, which is why more institutions should replicate the research. It was also possible to do further molecular analysis to determine the root cause of colistin resistance.

**Table 1: Demographic details of the patients with MDR NF-GNB Infections other than *Pseudomonas aeruginosa* and *Acinetobacter baumannii complex***

| Parameters | No. of cases (%) Cases of UTI=16 | No. of cases (%) Cases of skin and soft tissue=80 | No. of cases (%) Cases of BSI=42 | No. of cases (%) Cases of Respiratory tract infection=23 | No. of cases (%) Cases of sterile site infection=24 |
|---|---|---|---|---|---|
| Age-wise distribution | | | | | |
| 0–1 year | 0 | 0 | 3 | 3 | 5 |
| 1–18 year | 3 | 9 | 4 | 4 | 2 |
| 19–45 year | 4 | 15 | 5 | 6 | 8 |
| 45-65 year | 8 | 45 | 19 | 8 | 9 |
| >65 year | 1 | 2 | 11 | 2 | 0 |
| Gender | | | | | |
| Male | 9 (56.3%) | 43 (53.8%) | 25 (59.5%) | 15 (65.2%) | 13 (54.2%) |
| Female | 7 (43.8%) | 37 (46.3%) | 17 (40.5%) | 8 (34.7%) | 11 (45.8%) |
| Location of patient in hospital | | | | | |
| Intensive care unit | 8 | 33 | 21 | 9 | 14 |
| In-patient | 5 | 40 | 13 | 7 | 9 |
| Out-patient | 3 | 7 | 8 | 7 | 1 |

**Table 2: Pathogens isolated and clinical details of the patients**

| Characteristic | Urinary tract infection | Skin and soft-tissue infection | Bloodstream infection | Respiratory tract infection | Sterile site infection |
|---|---|---|---|---|---|
| Organism isolated | *Acinetobacter hemolyticus* 5 (31.3%)<br>*A. lwoffii* 11 (68.8%) | *Acinetobacter hemolyticus* 16 (20%)<br>*Acinetobacter lwoffii* 27 (33.8%)<br>*Alcaligenes* 3 (3.8%)<br>*Burkholderia cepacia* 2 (2.5%)<br>*Elizabethkingia meningoseptica* 1 (1.3%)<br>*Pseudomonas fluorescens* 15 (18.8%)<br>*Pseudomonas luteola* 2 (2.5%)<br>*Pseudomonas putida* 6 (7.5%)<br>*Pseudomonas stutzeri* 5 (6.3%)<br>*Stenotrophomonas maltophilia* 3 (3.8%) | *Acinetobacter hemolyticus* 7 (16.6%)<br>*Acinetobacter lwoffii* 22 (52.3%)<br>*Burkholderia cepacia* 4 (9.5%)<br>*Chryseobacterium indologenes* 1 (2.3%)<br>*Elizabethkingia meningoseptica* 1 (2.3%)<br>*Pseudomonas fluorescens* 1 (2.3%)<br>*Pseudomonas putida* 1 (2.3%)<br>*Stenotrophomonas maltophilia* 4 (9.5%)<br>*Raltsonia picketii* 1 (2.3%) | *Acinetobacter hemolyticus* 3 (13%)<br>*Acinetobacter lwoffii* 12 (52.2%)<br>*Burkholderia cepacia* 1 (4.3%)<br>*Alkaligenes* 1 (4.3%)<br>*Elizabethkingia meningoseptica* 1 (4.3%)<br>*Pseudomonas fluorescens* 1 (4.3%)<br>*Sphingomonas* 1 (4.3%)<br>*Stenotrophomonas maltophilia* 1 (4.3%)<br>*Raltsonia pickettii* 2 (8.6%) | *Acinetobacter hemolyticus* 3 (12.5%)<br>*Acinetobacter lwoffii* 11 (45.8%)<br>*Chryseobacterium indologenes* 1 (4.2%)<br>*Elizabethkingia meningoseptica* 2 (8.3%)<br>*Pseudomonas fluorescens* 2 (8.3%)<br>*Pseudomonas luteola* 2 (8.3%)<br>*Stenotrophomonas maltophilia* 3 (12.5%) |
| Clinical associates | CAUTI 7 (43.8%)<br>Pregnancy-ANC 4 (25%)<br>Renal stones 2 (12.5%)<br>Anatomical malformations-strictures, stenosis 2 (12.5%)<br>Others 1 (6.3%) | Post-operative wounds (orthopedics, post LSCS, post-surgical procedures) 63 (78.8%)<br>Ac. suppurative otitis media 4 (5%)<br>Ch. suppurative otitis media 13 (16.3%) | Post-operative wound sepsis 13 (30.9%)<br>Pneumonia 6 (14.2%)<br>Infective endocarditis 5 (11.9%)<br>Diabetes mellitus 12 (28.5%)<br>Hepatic encephalopathy 6 (14.2%) | Ac exacerbation COPD 5 (21.7%)<br>Cystic fibrosis 3 (13%)<br>Bronchial asthma 4 (17.4)<br>Pneumonia 9 (39.1%)<br>Pulmonary Koch's 2 (8.6%) | Peritoneal fluid (post op) 13 (54.2%)<br>Meningitis 6 (25%)<br>Arthritis 5 (20.8%) |

**Table 3: Susceptibility profile of MDR non-fermenters to various antimicrobials (% susceptibility)**

| Antimicrobial agents | *Acinetobacter hemolyticus* n (%) | *Acinetobacter Lwoffii* n (%) | *Burkholderia cepacia* n (%) | *Stenotrophomonas maltophilia* n (%) |
|---|---|---|---|---|
| Amikacin (30 µg) | 6 (17.6%) | 13 (15.4) | NT | NT |
| Cefepime (30 µg) | 1 (2.9%) | 5 (5.9) | NT | NT |
| Ceftazidime (30 µg) | 1 (2.9) | 4 (4.7) | 2 (28.5) | NT |
| Cefotaxime (30 µg) | 0 | 1 (1.1) | NT | NT |
| Ceftriaxone (30 µg) | 0 | 3 (3.5) | NT | NT |
| Ciprofloxacin (5 µg) | 4 (11.7) | 18 (21.4) | NT | NT |
| Gentamicin (10 µg) | 7 (20.6) | 20 (2.3) | NT | NT |
| Imipenem (10 µg) | 5 (14.7) | 9 (10.7) | NT | NT |
| Meropenem (10 µg) | 3 (8.8) | 10 (11.9) | 3 (42.8) | NT |
| Piperacillin-Tazobactam (110/10 µg) | 3 (8.8) | 17 (20.2) | NT | NT |
| Minocycline (30 µg) | 21 (61.7) | 51 (60.7) | 7 (100) | 3 (27.2) |
| Levofloxacin (5 µg) | NT | NT | NT | 5 (45.4) |
| Cotrimoxazole (1.25/23.75 µg) | NT | NT | 2 (28.5) | 4 (36.3) |
| Colistin (Intermediate<2 µg/mL) | 32 (94.11) | 84 (100) | NT | 9 (81.8) |

NT-not tested.

## CONCLUSION

With the dramatic increase of MDR Gram-negative bacteria, particularly non-fermenters, it is critical that practitioners in every area be informed about the most recent prevalence and pattern of antibiotic susceptibility of the circulating pathogens. Furthermore, because these bacteria may thrive in a hospital setting, better infection-control practices and antibiotic stewardship is necessary to stop or restrict the progression and spread.

## AUTHORS' CONTRIBUTIONS

Dr Mitisha Soni: Concept, design, literature search and data acquisition, and manuscript preparation. Dr. Garima Kapoor and Dr. Deepti Chaurasia: Data analysis, interpretation, and review. Dr Rajat Soni: Manuscript Editing. All authors: The approval of the final version of manuscript.

## CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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None.

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