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NON-FERMENTING BACTERIA: PREVALENCE AND ANTIMICROBIAL RESISTANCE TRENDS IN A TERTIARY CARE HOSPITAL IN ANDHRA PRADESH

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ABSTRACT:

INTRODUCTION: The increasing prevalence of non-fermenting bacteria in clinical settings poses a significant challenge to healthcare providers due to their intrinsic resistance to multiple antibiotics. These bacteria are often associated with severe infections and increased morbidity and mortality rates. The aim of the present study is to determine the prevalence of non- fermenting bacteria and analyze their antimicrobial resistance patterns in a tertiary care hospital in Andhra Pradesh.

MATERIALS AND METHODS: A retrospective cross-sectional study was conducted from September 2023 to September 2024 at the Department of Microbiology, KIMS, Amalapuram. Clinical samples (e.g., blood, urine, sputum, pus, BAL, blood, fluids) were collected aseptically from patients suspected of having bacterial infections and transported immediately to the microbiology laboratory for analysis. The samples were processed using standard microbiological techniques including Gram's stain and culture on selective media for bacterial identification and antibiotic susceptibility testing using Kirby Bauer Disc Diffusion method as per CLSI guidelines. Statistical analysis of the data was performed using SPSS software version 21.0

RESULTS: *Pseudomonas spp* (67.3%) were predominantly noted followed by *Acinetobacter species* (31.5%). Very few *Burkholderia spp* and *Stenotrophomonas maltophila* were isolated among clinical specimens accounting for 0.62% and 0.41% respectively. Carbapenem resistant NFGNB (Non-Fermenter Gram Negative Bacilli) infected patients showed prolonged length of stay and increased mortality rate significantly when compared to non MDR (Multi Drug Resistant) NFGNB infected patients. Non fermenters showed a sensitivity of 80% to meropenem, aminoglycosides, minocycline and the susceptibility towards beta lactam and beta lactamase inhibitor combination antibiotics varied among different non-fermenters.

CONCLUSION: By understanding the resistance patterns of non-fermenting bacteria, healthcare providers can make more informed decisions regarding antibiotic use, ultimately improving patient outcomes and reducing the spread of resistant strains. A strong focus on Infection control practices and Surveillance and Antimicrobial Stewardship programs by Central and State Health Authorities could largely contribute to controlling outbreaks or hospital acquired infections.

KEY WORDS: Non fermenter, Gram Negative Bacilli, Antimicrobial resistance

INTRODUCTION

A complex interplay of different predisposing factors such as patient comorbidities, steroid usage, prolonged length of stay, frequent hospital visits, medical interventions, bacterial pathogenicity, secondary infections, and treatment factors are causing emergence of Non fermenters as important pathogens. Non-Fermenters Gram negative bacilli like *Pseudomonas aeruginosa* and *Acinetobacter baumannii* are posing a major threat to health care settings as they have high potential for antimicrobial resistance. Most of these non-fermenters are multidrug resistant particularly in debilitating and immunocompromised hosts [1]. These non-fermenters can cause community acquired or hospital acquired infections either by direct transmission from patient to susceptible persons or by indirect transmission from reservoir to objects or contaminated surfaces without direct human to human contact [2].

The inherent antibiotic resistance patterns and treatment choices of non-fermenters are different from other medically important aerobic bacteria. So, knowledge on institutional antibiotic policies and antimicrobial trends can help clinicians to start empirical therapy and decrease the severity of infection. Antibiograms of the hospital are crucial tools that guide clinicians in selecting effective empirical therapy [3].

The increasing prevalence of non-fermenting bacteria in clinical settings poses a significant challenge to healthcare providers due to their intrinsic resistance to multiple antibiotics. These bacteria are often associated with severe infections, particularly in immunocompromised patients, and can lead to prolonged hospital stays, increased healthcare costs, and higher morbidity and mortality rates.[2]

Antimicrobial resistance was considered as “one of the threats to human health worldwide” by the Infectious Diseases Society of America (IDSA) [4]. Multidrug resistant organisms (MDRO) are bacteria that have become resistant to certain antibiotics, and these antibiotics can no longer be used to control or kill the bacteria. MDRO could be MRSA (Methicillin resistant *Staphylococcus aureus*), VRE (Vancomycin resistant *Enterococci*), CRAB (Carbapenem resistant *Acinetobacter baumannii*), *Pseudomonas aeruginosa* resistant to ceftazidime or carbapenems, Extended Spectrum Beta Lactamases (ESBL) producing Enterobacteriaceae. Other organisms were considered MDR if they were found to be resistant to at least three of the following antibiotic classes: antipseudomonal cephalosporins /penicillins, macrolides, carbapenems, fluoroquinolones, aminoglycosides, colistin, and tigecycline. Infections of multidrug resistant pathogens are becoming a major public health problem [5].

In Andhra Pradesh, there is limited data on the prevalence and antimicrobial resistance patterns of non-fermenting bacteria in tertiary care hospitals. This study aims to fill this gap by providing comprehensive data on the epidemiology and resistance profiles of these pathogens. The findings will help in antimicrobial stewardship programs, guide empirical therapy, and contribute to the development of effective infection control strategies.

Aim: To determine the prevalence of non-fermenting bacteria and analyze their antimicrobial resistance patterns in a tertiary care hospital in Andhra Pradesh.

Objectives:

To determine the prevalence of non-fermenting bacteria in the tertiary care hospital.

To isolate, identify the various species of non-fermenting bacteria present in different clinical samples and analyze their antimicrobial resistance patterns.

To assess the impact of antimicrobial resistance on patient outcomes and treatment efficacy.

MATERIALS AND METHODS

Study Design & Settings: A cross-sectional study conducted at a tertiary care hospital in Andhra

Pradesh. A total number of 1267 clinical isolates were collected from patients hailing from areas in and around Amalapuram from September 2023 to September 2024. This retrospective study was taken up after the review and approval by the IEC (Institutional Ethical Committee Lr.No.IEC/CD/2025).

Sample Collection: As per Central laboratory standard institute guidelines all the samples were collected. Clinical samples (e.g., blood, urine, sputum, pus, BAL, blood, fluids) were collected from patients suspected of having bacterial infections. Samples were collected aseptically as per the laboratory instructions and transported immediately to the microbiology laboratory for analysis.

Inclusion criteria:

1. Samples collected before the initiation of antimicrobial therapy.
2. All clinical samples including blood, urine, sputum, pus, broncho alveolar lavage, blood, body fluids sent to the Microbiology lab.

Exclusion criteria:

Mixed growth of 3 or more types (probably contaminated sample).

Isolation and Identification of Non-Fermenters:

All samples were processed for microscopic examination, culture and antibiotic susceptibility testing according to CLSI protocols. Specimens were inoculated onto nutrient agar, 5% sheep blood agar and Macconkey agar. After incubation at 37C for 24-48 hours colony count was done and expressed as the number of colony forming units per ml for BAL and urine samples. Pathogen identification up to species was performed by colony characterization, biochemical reactions and inoculation on special media. Bacterial growth with a colony $\geq 10^5$ cfu/ml (for urine), $\geq 10^5$ cfu/ml (for BAL) was considered as pathogens. The isolates which were non- lactose fermenting and showed alkaline change (K/NC) reaction in triple sugar iron agar media were provisionally considered as NFGNB. They were further identified based on motility, pigment production, colony morphology, Gram staining, Special media (eg. Cetrimide agar) and special biochemical tests (e.g. oxidase test, catalase test, Hugh-Leifson oxidative fermentative test for glucose, lactose, sucrose, maltose and mannitol, nitrate reduction test, indole test, citrate utilization test, urease test, utilization of 10% lactose, lysine and ornithine decarboxylation, arginine dehydrolation, growth at 42°C and 44°C.)[13]

Antimicrobial Susceptibility Testing:

Antimicrobial susceptibility of the isolated non-fermenters was determined using the Modified Kirby-Bauer disk diffusion method. The results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines.

Antibiotic discs procured from HiMedia used for Non fermenter isolates were: ceftazidime (30 µg), piperacillin+tazobactum (100/10 µg), ceftazidime (30 µg), Ceftazidime+clavulanic acid (30/10 µg), piperacillin+tazobactum (30/6 µg), cefaperazone+sulbactum (75/30 µg), minocycline (30 µg), levofloxacin (5 µg), meropenem (10 µg), tobramycin (10 µg), gentamicin (10 µg) and colistin (50 µg). Escherichia coli ATCC 25922 and Pseudomonas aeruginosa ATCC 27853 were used as control strains.[13]. Colistin susceptibility was determined using E-test and BD Phoenix M50 automated antimicrobial susceptibility system. Multi Drug Resistance testing was done for all strains isolated according to CLSI guidelines.

Data Collection:

The microbiological data was documented from the lab register and patient medical records were reviewed for patient demographic data, clinical features and patient outcomes like length of hospital stay and mortality were assessed. Patient confidentiality was maintained and the data was collected

anonymously.

Data Analysis:

The prevalence of non-fermenting bacteria and their antimicrobial resistance patterns were analysed using descriptive statistics. Comparisons were made between the resistance patterns of non-fermenters and other bacterial pathogens. Statistical analysis of data was performed using SPSS software version 21.0.

RESULTS

A total of 1267 clinical specimens were evaluated in this study. Out of these various clinical samples, 478 (37.7%) were identified as Non fermenters by conventional culture and sensitivity testing methods (Table 1). *Pseudomonas spp* (67.3%) were predominantly noted followed by *Acinetobacter species* (31.5%). Very few *Burkholderia spp* and *Stenotrophomonas maltophila* were isolated among clinical specimens accounting for 0.62% and 0.41% respectively.

Table 1. Organism wise distribution of Non fermenters

Organism Name	No. of isolates	Percentage
Pseudomonas spp	322	67.3%
Acinetobacter spp	151	31.5%
Burkholderia spp	03	0.62%
Stenotrophomas maltophila	02	0.41%
Total	478	100%

In this study non fermenters were isolated from various clinical isolates in which patients with respiratory infections were predominantly harbouring the non-fermenters when compared to urinary tract infection or wound infections. Sputum (34.7%) and BAL (26.1%) samples were the most common specimens with non-fermenters followed by Urine (12.1%) (Table 2).

Table 2. Non fermenters distribution in various clinical isolates

Specimen	*Ps.aeruginosa*	*Acinetobacter spp*	*Burkholderia*	*S.maltophili a*	Total	%
Sputum	97	65	3	1	166	34.7
BAL	85	40	0	0	125	26.1
Urine	75	19	0	0	94	19.6
Blood	15	8	0	0	23	4.8
Pus	42	15	0	1	58	12.1
Body fluids	8	4	0	0	12	2.5
Total	322	151	03	02	478	100%

Pseudomonas aeruginosa was <60% sensitive to all the first line antibiotics like: levofloxacin, cotrimoxazole, tetracycline, ceftazidime+clavulanic acid, cefoperazone+sulbactam, minocycline, nitrofurantoin and norfloxacin. 60-80% sensitive to second line antibiotic aminoglycosides - gentamicin, piperacillin+tazobactam and tobramycin, 80% sensitive to meropenem.
Acinetobacter spp was <60% sensitive to all the first line antibiotics like: levofloxacin, cotrimoxazole, tetracycline, ceftazidime+clavulanic acid, piperacillin+tazobactum, cefoperazone+sulbactam, nitrofurantoin and norfloxacin. 60-80% sensitive to second line antibiotic aminoglycosides - gentamicin, minocycline and 96% sensitive to tobramycin.
Burkholderia spp showed 80-90% susceptibility to cotrimoxazole, gentamicin, tobramycin, meropenem, and minocycline. *Stenotrophomonas maltophila* isolates were 80% sensitive to minocycline, around 75% were sensitive to levofloxacin, piperacillin+tazobactum, cefaperazone+sulbactum, ceftazidime+clavulanic acid (Table 3).

Table 3. Antibiotic susceptibility pattern of Non-fermenters

Organis m	CA Z	CT R	LE	TE	CO T	G	PIT	TO B	CF S	CX A	M RP	MI	NI T	CL
Pseudom onas	41%	IR	51 %	IR	IR	73 %	70 %	74 %	53 %	57 %	80 %	35%	21 %	100 %
Acientob acter	11%	12 %	25 %	40 %	31 %	68 %	40 %	96 %	47 %	37 %	41 %	63%	32 %	100 %
Burkhold eria	57%	IR	78 %	62 %	82 %	97 %	75 %	92 %	70 %	73 %	86 %	85%	-	IR
Stenotro phomona s	60%	IR	75 %	IR	38 %	IR	74 %	IR	73 %	75 %	IR	80%	-	100 %

*CAZ-Ceftazidime, CTR-Ceftriaxone, LE-Levofloxacin, COT-Cotrimoxazole, G-Gentamicin, PIT-Piperacillin+tazobactum, TOB-Tobramycin, CFS-Cefaperazone+sulbactum, CXA-Ceftazidime+clavulanic acid, MRP-Meropenem, MI-Minocycline, NIT-Nitrofurantoin, CL-Colistin.
Carbapenem resistant NFGNB infected patients showed prolonged length of stay and increased mortality rate significantly when compared to non MDR NFGNB infected patients.

Table 4. Outcome of Carbapenem resistant and Non MDR NonFermenter GNB infection

Outcome feature	Carbapenem resistant NFGNB (n=288)	Non MDR NFGNB (n=190)	OR	p value
Length of stay (IQR)	11 (7 to 14)	5 (3 to 7)	-	<0.001

Mortality	80 (16.7%)	23 (4.8%)	2.7926	0.0001

DISCUSSION

Acinetobacter and *Pseudomonas aeruginosa* are most commonly associated with device related infections; they are also commonly isolated in ICU settings [6,7]. *Acinetobacter* and *Pseudomonas infections* resistant to multiple drug classes including beta lactam/beta lactamase inhibitors might be due to enzymatic activation, alteration of target, acquiring resistance genes from other organisms, efflux pumps or porins, and biofilm formation.

In this study 37.7% (478/1267) isolates were non-fermenters. Most of the isolates were isolated from Sputum (34.7%) and BAL (26.1%) samples followed by Urine (12.1%). Our study showed high isolation rate which is similar to the study of Sidhu S et al [8] noted 45.9%. Few studies mentioned very low isolation rate of 3.58% and 5.2% [9,10]. Malini A et al observed 12-15% of Non-Fermenting Gram Negative bacilli (NF-GNB) isolates from clinical samples [11]. Grewal US et al noted non fermenters bacterial isolates were linked to osteomyelitis, septicaemia, pneumonia, urinary tract infection, ventilator-associated pneumonia and surgical site infections [12]. Ranjan Kumar et al [13] stated that total 332 NFGNB were isolated from 2157 culture positive clinical samples accounting for an isolation rate of 15.4%. Urine was the most common specimen obtained followed by pus and blood. Similar to our study, Sonia Agarwal et al [14] isolated NFGNB most commonly from ET aspirates i.e., 64.5%. Sarkar M et al [15] isolated NFGNB where Urine was the most common specimen (29.44%) followed by pus (27.49%), blood (15.57%), sputum (12.90%), tracheal aspirate (8.27%) and remaining 6.33% included other samples. Soni M et al [16] stated that MDR NF-GNB were predominantly isolated from pus (45.5%) followed by blood (20.5%), urine (12.4%), respiratory samples (11.7%), and sterile fluids (10%). Karvi Agarwal et al [17] noted 7.6% of non-fermenters in blood stream infections. Isolation rate of NFGNB from different clinical samples varies from region to region which could be due to microbiotia in the community, antibiotic usage in the region, percentage population presenting with different comorbidities.

Pseudomonas spp (67.3%) were predominantly noted followed by *Acinetobacter species* (31.5%). Very few *Burkholderia spp* and *Stenotrophomonas maltophila* were isolated among clinical specimens accounting for 0.62% and 0.41% respectively. Our study corroborated well with other studies which mentioned *P.aeruginosa* followed by *A.baumanii* [9,10,18]. Similar to this study Jitendranath et al [19] and Juyal et al [20] observed *Pseudomonas aeruginosa* as the predominant pathogen accounting for 57.7% and 38.21% respectively. Ranjan Kumar et al
[13] noted *A. baumannii* was the most common species isolated, accounting for 52.1%, followed by *P. aeruginosa* (40.1%) and *B. cepacia* complex (5.7%). Rest was constituted by
A. lwoffii, B. pseudomallei and *S. maltophilia* together. Goel V et al [21] noted *A. baumannii* (48.78%) was the most commonly isolated pathogen followed by *P. aeruginosa* (37.71%). According to Samanta P et al [22], the isolation rate of *Acinetobacter species* was 66%, and *Pseudomonas species* was 26%. Sharma S et al [23] *Acinetobacter calcoacetecus baumanii* complex 67(46.8%) was the commonest isolate followed by *Pseudomonas aeruginosa* 56(39.1%). While other NFGNB like *Elizabethkingia, Stenotrophomonas, Sphingomonas, Burkholderia* and *Chrysobacterium* were isolated in few respiratory samples.

Non fermenters showed a sensitivity of 80% to meropenem, aminoglycosides, minocycline and the susceptibility towards beta lactam and beta lactam antibiotics varies among different non-fermenters. *Pseudomonas* and *Acinetobacter* were <60% sensitive to cefoperazone+sulbactam, piperacillin+tazobactam, and ceftazidime+clavulanic acid in the present study. Ranjan kumar et al [13] did a study on non-fermenters antibiotic susceptibility testing in which *A.baumanii* and *Pseudomonas aeruginosa* showed highest sensitivity to gentamicin and amikacin & lower sensitivity to ceftriaxone. *B. cepacia complex, B. pseudomallei* and *S. maltophilia* showed 100% susceptibility to cotrimoxazole. If *Acinetobacter* exhibits carbapenem resistance then clinicians are left with few antibiotics to manage an infection. Gokale S et al [24] showed highest susceptibility to

meropenem (96.2%) and 45% susceptibility to ciprofloxacin for *A. baumannii*. *P. aeruginosa* showed highest susceptibility to amikacin, but least susceptibility to ceftriaxone. The Centers for Disease Control and Prevention reported 32,600 cases of multidrug-resistant (MDR) *Pseudomonas aeruginosa* infection from hospitalized patients in the United States in 2017, causing 2,700 deaths [25]. MDR *Acinetobacter baumannii* were highly susceptible to colistin (97.7%) and minocycline (64.4%) but poorly susceptible to ampicillin (16.1%) and ceftazidime (6.9%). In a study on non-fermenters from lower respiratory tract infections, all *Acinetobacter* isolates were MDR and were only susceptible to colistin (100%) [19]. Similar results were obtained by Nazir et al study, where *Burkholderia cepacia* complex showed high susceptibility to minocycline (70%) followed by meropenem (67.7%) [20]. However, the studies by Sarkar et al [15] found 100% susceptibility to cotrimoxazole. *Stenotrophomonas maltophilia* was susceptible to colistin (90.9%), followed by cotrimoxazole (36.4%), minocycline (27.3%), and levofloxacin (27.3%). *Sonia Agarwal et al* [14] observed that 90.5% *Acinetobacter baumannii* were resistant to imipenem and 95.2% resistant to meropenem, *Pseudomonas aeruginosa* came out to be 52% resistant to imipenem and 56% resistant to meropenem while *Stenotrophomonas maltophilia* and *Elizabethkingia meningoseptica* were 100% resistant to carbapenems as they are intrinsically resistant to carbapenems. The study by Sarkar et al [15], Nazir et al [26] and Juyal et al [20] also showed high susceptibility to cotrimoxazole. A study on MDR NFGNB from Central India by Soni M et al [16] noted multi drug resistant pathogens which were resistant to almost all antibiotics except colistin. *Pseudomonas aeruginosa* and *Acinetobacter species* showed 94.8% and 98% sensitivity to Colistin respectively. *Burkholderia cepacia* complex was 100% susceptible to minocycline and 28.6% susceptible to ceftazidime. Out of 11, 10 (90.9%) *Stenotrophomonas maltophilia* were susceptible to colistin and 27.3% susceptible to ceftazidime and minocycline (27.3%). Sharma S et al [23] observed the most effective antibiotics were Tigecycline and Polymyxin B/ Colistin, however majority of them showed multidrug resistance. Al-Kadmy IM et al [27] noted as all 33 colistin resistant strains (MIC $\geq$ 4 mcg/mL by broth microdilution) were found to be negative for the most frequently studied plasmid-mediated mcr-1, mcr-2, and mcr-3 genes. *Acinetobacter baumannii* and *Pseudomonas aeruginosa* are well established health care associated pathogens due to their ability to develop resistance to various antibiotics including carbapenems by various mechanisms [11].

Carbapenem resistant NFGNB infected patients showed prolonged length of stay and increased mortality rate significantly when compared to non MDR NFGNB infected patients in the present study. Prolonged hospital stay, instrumentation, burns, surgical site infections, prematurity (in case of neonates), diabetes, malignancies, and various underlying ailments predispose these patients to NF-GNB infections [11]. Mechanism of carbapenem resistance in non-fermenting Gram-negative bacilli occurs by various mechanisms including carbapenemase production, decrease permeability due to loss of porin channels, overexpression of efflux pump and changes in penicillin binding proteins [28].

CONCLUSION

Globally, nosocomial infections are accounting for significant increase in morbidity and mortality, out of which non-fermenter Gram negative bacilli play a critical role in device associated infections. This is due to their ability to survive in hospital environment including disinfectants and increase in antimicrobial resistance by their genetic potentiality and acquiring drug resistance genes.

By understanding the resistance patterns of non-fermenting bacteria, healthcare providers can make more informed decisions regarding antibiotic use, ultimately improving patient outcomes and reducing the spread of resistant strains. A strong focus on Infection control practices and Surveillance and Antimicrobial Stewardship programs by Central and State Health Authorities could largely contribute to controlling outbreaks or hospital acquired infections

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