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Original Research Article

DISTRIBUTION AND ANTIMICROBIAL SUSCEPTIBILITY OF NONFERMENTING GRAM-NEGATIVE BACTERIA FROM CLINICAL SPECIMENS AT A TERTIARY CARE TEACHING HOSPITAL IN NORTH KERALA

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ABSTRACT

Background: Nonfermenting Gram-negative bacteria (NFGNB), chiefly Pseudomonas aeruginosa and Acinetobacter baumannii, are frequently isolated in hospital laboratories in India. Their relative proportions and antibiograms differ from one centre to another, so each hospital needs its own data. The objective is to study the distribution of NFGNB across specimen types, hospital departments and age and sex groups, and their reported antimicrobial susceptibility, in a tertiary care teaching hospital in North Kerala.

Materials and Methods: Data were taken from the results tables of a cross-sectional study done in the Department of Microbiology between August 2022 and July 2023. Isolates were identified by conventional methods, and VITEK 2 was used when biochemical tests were inconclusive. Susceptibility testing was by Kirby–Bauer disc diffusion. We recalculated counts and percentages from the source tables but used the susceptibility percentages as reported.

Results: Out of 13,549 specimens processed, 732 NFGNB were included, i.e. 5.40 isolates per 100 specimens. The specimen table listed 3,151 culture-positive specimens, and the ratio of included isolates to culture-positive specimens was 23.23%. P. aeruginosa (572, 78.14%) was the commonest isolate, followed by A. baumannii (126, 17.21%). Pus (269, 36.75%), sputum (204, 27.87%) and urine (151, 20.63%) were the main sources. Reported susceptibility of P. aeruginosa to piperacillin–tazobactam, amikacin and meropenem was 94.8%, 93.2% and 92.9%, respectively, compared with 41.2%, 48.3% and 36.4% for A. baumannii.

Conclusion: P. aeruginosa was the predominant NFGNB in this collection, and A. baumannii was much less susceptible to every agent studied. Species-wise surveillance is recommended, but because of gaps in the source data the percentages should not be used as a validated clinical antibiogram.

Keywords: Nonfermenting Gram-negative bacteria; Pseudomonas aeruginosa; Acinetobacter baumannii; antimicrobial susceptibility; clinical specimens; Kerala.

INTRODUCTION

Nonfermenting Gram-negative bacteria (NFGNB) are aerobic, non-sporing bacilli or coccobacilli that do not ferment carbohydrates. They either do not use them at all or break them down by the oxidative route. At the bench, the usual first clue is a colony that fails to produce acid on triple sugar iron agar. Most of these organisms live in soil, water and moist hospital sites such as sinks, humidifiers and respiratory equipment, and several are intrinsically resistant to many antibiotics owing to low outer-membrane permeability, chromosomal efflux pumps and inducible β-lactamases. Once dismissed as

contaminants, NFGNB are now accepted as important healthcare-associated pathogens.[1-9]

Among them, P. aeruginosa and A. baumannii are clinically the most important. In the global estimate of the burden of bacterial antimicrobial resistance for 2019, both were among the six leading pathogens responsible for deaths associated with resistance.[1] In India, the 2024 annual report of the ICMR Antimicrobial Resistance Research and Surveillance Network also identified these two species as the main nonfermenters in its tertiary care centres.[2]

Hospital-based studies from India show considerable variation. Choudhury et al. from Kolkata and Soni et al. from central India described a wide range of NFGNB with differing susceptibility patterns.[3,4] Part of this difference depends on how the study population and the denominator were defined, whether all clinical samples, culture-positive samples or only multidrug-resistant isolates.

Other workers have looked at selected groups, such as bloodstream infections,[5] adult lower respiratory tract infections,[6] and neonatal sepsis.[7] Findings from these groups cannot be applied directly to the full range of specimens received in a hospital laboratory, and an institutional report is easier to interpret when the specimen mix and patient profile are given along with the organism-wise results.

The resultsare based on the clinical microbiology component of a project conducted at MES Medical College, Perinthalmanna. Our objectives were to describe the distribution of NFGNB isolates by specimen type and hospital department, to summarise their age and sex distribution, and to present the reported phenotypic susceptibility of the predominant species. All study data come from the thesis, and recent literature has been used only for comparison in the discussion. Molecular resistance testing is outside the scope of this report.

MATERIALS AND METHODS

Study design and setting: The source study was a cross-sectional investigation conducted in the Department of Microbiology, MES Medical College, Perinthalmanna, Kerala, between 1 August 2022 and 31 July 2023. The thesis describes convenience sampling and records 13,549 clinical specimens processed during this period. We drew only on the aggregate results reported in the thesis and introduced no additional patients, specimens or laboratory measurements.

Selection of isolates and terminology: The study included NFGNB isolates judged to be pathogens on clinical correlation and excluded isolates from repeat samples of the same patient. The thesis does not, however, set out a detailed clinical adjudication rule or a deduplication interval. The recorded collection comprised 732 isolates from pus, urine, sputum, blood, bronchoalveolar lavage, bronchial wash and endotracheal aspirate.

We retained the original collection in full. It contained two isolates reported as Moraxella catarrhalis, an asaccharolytic Gram-negative diplococcus, and for this reason the broader term “nonfermenting Gram-negative bacteria” is used throughout. Ten isolates recorded as Flavobacterium were kept at genus level as Flavobacterium spp. Other organism names follow the identifications in the results tables, with spelling corrected. No further species confirmation was assumed.

Culture and identification: Specimens were collected with aseptic precautions and processed according to the procedures described in the thesis. Gram-stained smears were examined for pus cells and bacteria. The laboratory inoculated specimens onto 5% sheep blood agar and MacConkey agar, with urine additionally plated on cystine–lactose–electrolyte-deficient agar. Plates were incubated at 37°C for 18–24 hours and then examined for growth. Preliminary identification relied on colony morphology, pigmentation, Gram reaction, motility, and catalase and oxidase reactions. Colonies that failed to acidify triple sugar iron agar were worked up as presumptive nonfermenters, with further biochemical characterisation that included carbohydrate utilisation and other differentiating tests. Isolates that conventional methods could not identify were tested on the VITEK 2 Compact system using Gram-negative identification cards, after suspensions had been adjusted to 0.5 McFarland with the VITEK 2 DensiCHEK instrument. Automated identification served only as a supplementary step. The study was not designed as a paired comparison of conventional and VITEK 2 methods.

Antimicrobial susceptibility testing: Susceptibility was tested by Kirby–Bauer disc diffusion on Mueller–Hinton agar using commercially available discs (HiMedia, Mumbai). Zone diameters were interpreted against the 2022 CLSI standard cited in the source study [8]. For the two predominant organisms, the reported panel comprised piperacillin–tazobactam, cefepime, ceftazidime, gentamicin, amikacin, ciprofloxacin, levofloxacin, imipenem and meropenem.

We took susceptibility percentages directly from the organism-specific results tables. The corresponding tested denominators, complete intermediate-category counts and raw zone diameters were not available. We therefore did not back-calculate susceptible counts, did not derive resistance as 100% minus susceptibility, and did not estimate the prevalence of multidrug resistance. Polymyxin results and the antibiograms for uncommon organisms were omitted because their testing or interpretation could not be adequately established from the source. We also excluded the separate combined carbapenem-susceptibility classification, which did not reconcile with the drug-specific tabulation.

Data handling and statistical analysis: Percentages for organism, sex, age and departmental distributions were recalculated with 732 isolates as the denominator. The component counts of the

specimen-wise table summed to 3,151 culture-positive submissions, and this figure served as the denominator for the culture-positive summary. Other narrative totals were not substituted.Overall isolate yield was calculated as 732 divided by 13,549, multiplied by 100. For specimen-specific yield, we divided the number of included isolates by the number of submissions in each specimen category. These figures are ratios of included isolates to specimens processed.

Ethics: The study was approved by the Institutional Ethics Committee, MES Academy of Medical Sciences (IEC/MES/21/2022). Informed consent was obtained from all patients included in the study.

RESULTS

Specimen processing and isolate yield: A total of 13,549 specimens were processed during the study period. Specimen-wise culture-positive counts totalled 3,151, a culture positivity of 23.26%. The 732 included nonfermenting isolates correspond to 5.40 isolates per 100 submissions and, relative to the tabulated culture-positive denominator, to an isolate-to-positive-specimen ratio of 23.23%.

Pus provided the largest share of isolates, 269 (36.75%), followed by sputum with 204 (27.87%) and urine with 151 (20.63%); together these three categories accounted for 624 isolates (85.25%). Blood contributed 49 (6.69%), bronchoalveolar lavage 38 (5.19%), bronchial wash 20 (2.73%) and endotracheal aspirate one (0.14%) [Table 1].

Submission volume did not track isolate yield. Urine accounted for 5,644 submissions yet gave only 151 included isolates, or 2.68 per 100 submissions, against 9.19 for pus and 13.11 for sputum. The highest yield came from bronchoalveolar lavage: 23.31 per 100 submissions, from 38 isolates in 163 specimens [Figure 1]. Only 12 endotracheal aspirates were received, yielding a single isolate.

Organism distribution: P. aeruginosa accounted for 572 isolates (78.14%) and A. baumannii for 126 (17.21%). Between them, the two species made up 95.36% of the collection. The remaining organisms were Burkholderia cepacia, 12 (1.64%); Flavobacterium spp., 10 (1.37%); Stenotrophomonas maltophilia, eight (1.09%); M. catarrhalis, two (0.27%); and Chryseobacterium indologenes and Burkholderia pseudomallei, one each (0.14%). Figure 2 shows the complete distribution.

Demographic and departmental distribution: Male patients contributed 450 isolates (61.48%) and female patients 282 (38.52%), a male-to-female ratio of 1.60:1. Patients older than 60 years accounted for 424 isolates (57.92%) and those aged 51–60 years for 147 (20.08%); all other recorded age groups together contributed 161 (21.99%).

Medicine was the largest contributor, with 319 isolates (43.58%), followed by Surgery with 167 (22.81%) and the intensive care units with 161 (21.99%). Orthopaedics accounted for 37 isolates (5.05%) and Obstetrics and Gynaecology for 21 (2.87%). The other recorded locations together supplied 27 isolates (3.69%) [Table 2]. These proportions describe the composition of the isolate collection. They do not estimate departmental infection rates.

Reported antimicrobial susceptibility: For every agent listed in [Table 3], reported susceptibility was higher in P. aeruginosa than in A. baumannii. Among P. aeruginosa isolates, reported susceptibility was 94.8% to piperacillin–tazobactam, 93.2% to amikacin, 92.9% to meropenem and 90.5% to imipenem. Ceftazidime, cefepime, gentamicin and ciprofloxacin followed at 87.8%, 84.0%, 83.7% and 83.4%, respectively. The table also reported 100% susceptibility to levofloxacin but did not state how many isolates had been tested.

A. baumannii was most often susceptible to amikacin, at 48.3%. Values for the other agents were 41.2% for piperacillin–tazobactam, 40.9% for imipenem, 39.5% for gentamicin, 37.0% for levofloxacin, 36.4% for meropenem and 31.9% for ciprofloxacin. Ceftazidime (24.1%) and cefepime (15.9%) had the lowest reported values. These figures reproduce the source tabulation; we did not reclassify them independently from raw measurements.

Table 1: Specimen submissions and included nonfermenting isolates

Specimen	Processed n	Culture positive n	Included isolates n	Share of 732 %	Yield per 100
Pus	2,927	1,311	269	36.75	9.19
Urine	5,644	1,133	151	20.63	2.68
Sputum	1,556	366	204	27.87	13.11
Blood	3,132	210	49	6.69	1.56
Endotracheal aspirate	12	9	1	0.14	8.33
Bronchoalveolar lavage	163	95	38	5.19	23.31
Bronchial wash	115	27	20	2.73	17.39
Total	13,549	3,151	732	100.00	5.40

Table 2: Demographic and departmental distribution of included isolates

Category	Isolates n	Percentage
Male	450	61.48
Female	282	38.52
Age older than 60 years	424	57.92
Age 51–60 years	147	20.08
Other recorded age categories	161	21.99
Medicine	319	43.58

Surgery	167	22.81
Intensive care units	161	21.99
Orthopaedics	37	5.05
Obstetrics and Gynaecology	21	2.87
Other recorded departments	27	3.69

Table 3: Reported susceptibility of the two predominant organisms

Antimicrobial	P. aeruginosa Reported S %	A. baumannii Reported S %
Piperacillin–tazobactam	94.8	41.2
Cefepime	84.0	15.9
Ceftazidime	87.8	24.1
Gentamicin	83.7	39.5
Amikacin	93.2	48.3
Ciprofloxacin	83.4	31.9
Levofloxacin	100.0	37.0
Imipenem	90.5	40.9
Meropenem	92.9	36.4

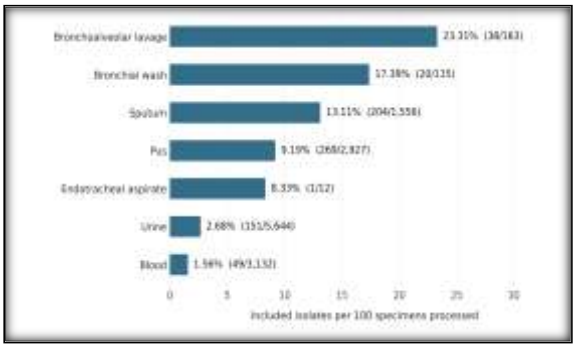

Figure 1: Isolate yield within each specimen category

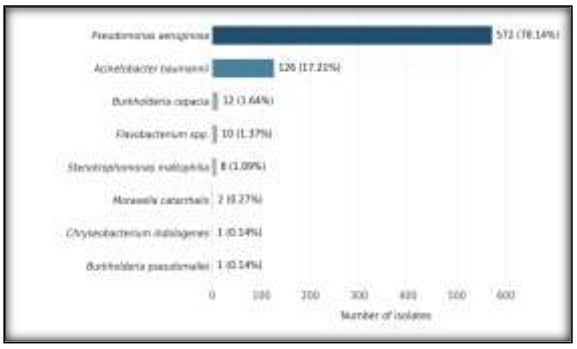

Figure 2: Distribution of reported organisms in the 732-isolate collection

DISCUSSION

P. aeruginosa made up 78.14% of the recorded isolates and A. baumannii 17.21%, together 95.36% of the collection. The Kolkata study also found Pseudomonas spp. and Acinetobacter spp. to be its two largest groups, but in more even proportions, 49.7% and 43.2%, respectively.[3] The ICMR 2024 surveillance collection was similarly balanced, with P. aeruginosa at 50.0% and A. baumannii at 45.3% of nonfermenters.[2] The strong predominance of P. aeruginosa here should be read as a local finding rather than as an estimate for North Kerala. Case mix, specimen selection and the level of identification all differ between these collections, and each limits direct comparison.

In this study, the yield of NFGNB was 5.40 per 100 specimens processed, and the ratio of included isolates to the culture-positive specimens listed in the table was 23.23%. The second figure is close to the 23.31% reported by Choudhury et al. from Kolkata, but their value was calculated against all bacterial isolates.[3] Soni et al. isolated 2,106 NFGNB from 21,019 culture-positive samples, of which 743 (35%) were multidrug-resistant, and their organism-wise analysis was confined to these 743 isolates.[4] Because each study used a different denominator (all specimens, culture-positive specimens, all bacterial isolates or resistant isolates alone), the percentages cannot be compared directly as prevalence.

Pus was the commonest source of NFGNB in our study, followed by sputum and urine, and pus was also the leading specimen in the Kolkata series.[3] Among the multidrug-resistant non-fermenters of Soni et al., pus (45.50%) again came first, followed by blood (20.50%).[4] Yield per specimen type was quite different: urine made up the largest number of submissions (5,644) but gave only 2.68 isolates per 100 specimens, against 13.11 for sputum and 23.31 for bronchoalveolar lavage (BAL). The high BAL yield is probably related to bronchoscopic samples being sent mostly from ventilated or seriously ill patients, though the thesis did not record the indications. In Bhubaneswar, where Choudhury et al. studied only BAL fluid and endotracheal aspirates from adults with lower respiratory tract infection, NFGNB grew in 757 of 2,389 patients (31.69%) and A. baumannii was isolated more often than P. aeruginosa.[6] A different study population may explain this, but our data cannot confirm it. Isolate counts and specimen-wise yields also do not tell us how often infection actually occurred.

More than three-fourths of the isolates (571, 78.01%) came from patients older than 50 years, and males outnumbered females by 1.60:1. Medicine, Surgery and the intensive care units together accounted for 647 isolates (88.39%). These are features of the isolates received in the laboratory and not independent risk factors, since population denominators and exposure data were not available. Selected groups behave differently. Panda et al., studying neonatal sepsis, found NFGNB in 48 (29%) of 168 blood culture isolates, 70.8% of them multidrug-resistant,[7] a setting very different from our largely elderly, mixed-specimen population.

Likewise, the large share from intensive care units does not by itself prove healthcare-associated acquisition, as admission dates and clinical details were not available.

The remaining organisms were few, 34 isolates (4.64%) in all, and no stable susceptibility estimate can be drawn from such small numbers. In respiratory samples from Manipal, Chawla et al. also found NFGNB other than P. aeruginosa and Acinetobacter spp. to be uncommon (33 isolates, 4%), with S. maltophilia the most frequent, and 23 (69.7%) of them were judged pathogenic after clinical correlation.[9] The same caution applies to organisms such as C. indologenes, which often colonises rather than infects. In our collection, the ten Flavobacterium isolates were identified only up to genus level and two isolates were M. catarrhalis, which is why the broader term "nonfermenting Gram-negative bacteria" has been used. The single isolate of B. pseudomallei deserves mention. Melioidosis is being reported more often from coastal South India, and an oxidase-positive bacillus with dry, wrinkled colonies on prolonged incubation and resistance to gentamicin and colistin should raise suspicion of this organism, keeping in mind the biosafety risk to laboratory staff.

For these minor groups, organism-specific studies are more informative. Suhartono et al. reported B. cepacia from a range of clinical specimens in an Indonesian tertiary hospital, including blood and sterile body fluids,[10] and Namaji et al. studied 42 pure-growth isolates of S. maltophilia from Pune.[11] In 41 patients with S. maltophilia from Oman, Sannathimmappa et al. found that 73% were male, all inpatients had at least one comorbidity, and 44% had received intensive care and 41% mechanical ventilation. Susceptibility was highest to minocycline (97%) and lowest to ceftazidime (50%).[12] Over a decade (2007–2016) at Chandigarh, Sethi et al. observed increasing resistance of the B. cepacia complex to ceftazidime, meropenem and minocycline, whereas S. maltophilia remained more than 80% susceptible to minocycline, levofloxacin and co-trimoxazole.[13] No comparable clinical information was available for our 12 B. cepacia and eight S. maltophilia isolates. These organisms are better followed by separate long-term surveillance than by pooled NFGNB percentages.

The two main species differed widely in reported susceptibility. Reported susceptibility of P. aeruginosa was above 80% for every agent tested, whereas no value for A. baumannii exceeded 50%, amikacin (48.3%) being the most active and cefepime (15.9%) the least. Madhavi et al. also found extensive resistance among clinical Acinetobacter isolates in Karnataka,[14] although their genus-level data and the incomplete susceptibility records in our source make a direct numerical comparison difficult. The poor activity of cephalosporins against A. baumannii here (ceftazidime 24.1%, cefepime 15.9%) agrees with Sarwat et al., who found cephalosporins ineffective against NFGNB from blood while colistin, carbapenems and fluoroquinolones generally remained active.[5] In Indian A. baumannii, carbapenem resistance is frequently attributed to acquired OXA-type carbapenemases, particularly OXA-23, often together with increased efflux. Molecular tests were not done in this study, so no mechanism can be assigned to our isolates.

The high susceptibility reported for P. aeruginosa needs cautious interpretation. The ICMR report for 2024 recorded 69.3% susceptibility to piperacillin–tazobactam, 73.3% to amikacin and 62.0% to meropenem,[2] well below the 94.8%, 93.2% and 92.9% in the thesis. The period, centres and patients are not the same, so this is not a like-for-like comparison. Even so, the gap is wide, and the testing denominators and interpretive steps should be checked locally before these figures are used to guide prescribing. Disc diffusion results for P. aeruginosa are affected by inoculum density, agar depth and disc potency, and without quality-control records a technical contribution cannot be ruled out.

Some agents need MIC-based methods. Sengupta and Banerjee determined sulbactam and colistin MICs for respiratory Acinetobacter isolates by E-strip and broth microdilution, and found the isolates susceptible to colistin but highly resistant to sulbactam.[15] Soni et al. tested colistin by broth microdilution and found that none of their 33 colistin-resistant strains carried the plasmid-mediated mcr genes screened by PCR.[4] Broth microdilution is the reference method for polymyxins, as these large cationic molecules diffuse poorly through agar. Polymyxin testing in the thesis could not be confirmed to this standard, so those results were left out, and published data cannot take their place.

For these reasons, the susceptibility data here carry a narrower meaning than a verified hospital antibiogram. Drug-wise denominators and raw zone diameters were not available, so the interpretive categories could not be rechecked. The 100% levofloxacin figure for P. aeruginosa, for instance, may not represent all 572 isolates. Since intermediate and missing results were not known, the percentage resistant cannot be obtained by subtraction, and we have not attempted an empirical treatment ranking or an estimate of multidrug resistance.

VITEK 2 was used only for isolates that conventional tests could not identify, and as it was not run in parallel on every isolate, agreement between the two methods could not be assessed.

Limitations: This was a single-centre study with convenience sampling, and the 2022–2023 data should not be taken as a current regional antibiogram. Repeat isolates were excluded as per protocol, but the deduplication rule and the criteria for clinical significance were not clearly described. Clinical outcome, prior antibiotic exposure and device-related data were not available for analysis.We could not confirm the distinction between species and species-complex identifications, the drug-wise denominators, the quality-control records for susceptibility testing or the use of organism-specific breakpoints. The conflicting specimen cross-tabulation and the

combined carbapenem classification were therefore excluded. These gaps chiefly affect the clinical use of the susceptibility data.

CONCLUSION

P. aeruginosa and A. baumannii made up most of the NFGNB in this collection, and pus, sputum and urine were the main sources. The corrected yield was 5.40 isolates per 100 specimens processed. A. baumannii had lower reported susceptibility than P. aeruginosa to all the selected agents. These findings support species-wise surveillance of NFGNB in our hospital, but inconsistencies in the data and incomplete susceptibility records limit their use for guiding empirical therapy.

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