Prevalence and susceptibility pattern of *Acinetobacter baumannii* and *Pseudomonas aeruginosa* from various clinical samples at a tertiary care center in Chennai, Tamil Nadu

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Abstract
Given their severity, inherent antibiotic resistance and potential to develop novel drug resistance, A. baumannii and P. aeruginosa infections are of utmost relevance among Gram-negative bacterial infections. Here, we studied the prevalence of A. baumannii and P. aeruginosa and their antibiotic susceptibility profile in a tertiary care center. A prospective study was conducted for clinical samples received at the Department of Microbiology, SRM Medical College Hospital and Research Centre between September 2021 and February 2022. The various clinical samples studied were blood, pus, urine, tissue, ear swab, vaginal swab and sputum. All the samples were processed according to standard conventional methods and non-fermenting Gram-negative bacteria obtained from these samples were included in the study. On microbiological analysis, most of the isolates were identified to be P. aeruginosa (72%) and A. baumannii (28%).

The overall prevalence was higher among males 64% compared to females 36% in the age group of 46 – 60 years. The prevalence of MDR phenotype was high in A. baumannii (60%) compared to P. aeruginosa isolates (14%) in this study. This study shows that the MDR phenotype of A. baumannii is more prevalent in intensive care units. The emergence of colistin resistance among the study isolates is concerning. This suggests that the availability of local susceptibility data is essential to aid in the selection of appropriate antimicrobial drugs for effective treatment. Thus, the appropriate use of antibiotics is necessary to prevent the emergence of antimicrobial resistance.

Keywords: *A. baumannii*, *P. aeruginosa*, Gram-negative bacteria, Multi-drug resistance.

Introduction

Infections with Gram-negative bacteria (GNBs) rank among the most serious health issues that affect both community and hospitalized patients. Because of the lipopolysaccharide layer (LPS), GNBs are known to cause sepsis more frequently, which increases patient morbidity and mortality[1]. Enterobacteriaceae and the non-fermenting Gram-negative bacteria (NFGNB) are the two major groups responsible for most clinical cases of Gram-negative infections. Among them, non-fermenters are extremely important because of the serious infections they can cause and their inherent resistance to most antibiotics, even if their proportion of infections is lower than that of Enterobacteriaceae[2,3].

They contribute to various complications like bloodstream infections, urinary tract infections, pneumonia and skin and and soft tissue infections[4].

Acinetobacter spp and *Pseudomonas spp* are the two most prominent pathogens causing infections that are clinically significant among non-fermenters. These species exhibit inherent resistance to multiple widely used antibiotics and they can acquire resistance to broad-spectrum β-lactams, aminoglycosides, fluoroquinolones and tetracyclines. Due to their pervasiveness in the intensive care unit and insufficient infection control procedures, their prevalence has steadily increased[2]. The other commonly isolated NFGNBs from clinical samples are *Stenotrophomonas maltophilia, Burkholderia spp, Moraxella spp* and *Ralstonia spp*[5].

The increasing incidence of multidrug resistance (MDR), a significant healthcare issue, is the primary concern of NFGNB. Notably, MDR *P. aeruginosa* and carbapenem-resistant *Acinetobacter baumannii* (CRAB) are classified as critical priority pathogens for which new antimicrobials are urgently needed by the CDC (Centre for Disease Control and Prevention)[4,5]. Many studies are available on the prevalence and resistance pattern of these pathogens. However, resistance rates may vary among individuals, geographic regions and hospital settings.

It may be possible to reduce treatment failure and costs by understanding the spectrum and resistance trends to inform successful empirical antibiotic therapy. The prevalence and antimicrobial susceptibility pattern of NFGNB are only partially provided by Indian studies.

The present study documents the prevalence of drug-resistant *Acinetobacter spp* and *Pseudomonas spp* among ICU (intensive care units) patients in a tertiary care hospital in Chennai, Tamil Nadu. The study also aims to determine the antimicrobial resistance among isolates from different clinical specimens.

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Material and Methods

Study design: This prospective study was carried out for 6 months from September 2021 to February 2022 in the Department of Microbiology, SRM Medical College Hospital and Research Centre, Kattankulathur campus, Chengalpattu. The study includes clinical samples received from the ICU (Intensive Care Unit) at the microbiology laboratory for routine culture and antibiotic susceptibility testing. This includes samples such as blood, pus, urine, tissue, ear swab, vaginal swab and sputum from all age groups.

Ethical approval for research: The study was approved by the Institutional Ethical Committee of SRMMCH and Research Center with reference number 8447/IEC/2022.

Laboratory methods: For microbial isolation, the samples were subjected to microscopy, Gram staining and inoculation onto blood agar and MacConkey agar and incubated aerobically at 37°C for overnight. The isolates were identified using standard methods based on the colony morphology, Gram staining, catalase, oxidase and motility tests. Conventional biochemical tests were done to isolate *Pseudomonas aeruginosa* and *Acinetobacter baumannii*[8]. Further, the species identification was confirmed by MALDI-TOF (Bruker Daltonik GmbH, Bremen, Germany). Other NFGNB isolates were excluded from this study.

The antimicrobial susceptibility of the isolates was evaluated using the conventional Kirby–Bauer disk diffusion method and interpreted according to the CLSI 2021 guidelines [9]. The antibiotics tested were as follows: Ceftazidime, Cefepime, Piperacillin/Tazobactam, Ciprofloxacin, Levofloxacin, Imipenem, Meropenem, Amikacin, Gentamicin, Tobramycin, Co-trimoxazole, Colistin and Polymyxin B. Multidrug-resistant (MDR) was defined as isolate exhibiting resistance to three or more distinct classes of antibiotics. A pan-resistant isolate was resistant to all commonly used antibiotics.

Statistical Analysis: All the data was maintained and analyzed in Microsoft Excel 2021. Descriptive data was analyzed as percentages and presented as graphs and tables. The data analysis was done using SPSS software version 25.0.

Results

From a total of 3230 non-duplicate samples collected during the study period, NFGNB was isolated from 88 samples. Of them, 72% (n=63) and 28% (n=25) of the isolates were identified to be *P. aeruginosa* and *A. baumannii* respectively. The overall prevalence was higher among males 64% (n=56) compared to females 36%(n=32). The maximum number of *P. aeruginosa* and *A. baumannii* was isolated from the 46-60 years (26%) age group (Figure 1) whereas 22% (n=19) were between the age group of 61-75 years, 20% (n=18) were between the age group of 16-30 years, 19% (n=17) were between the age group of 31-45 years, less than 10% of isolation was observed among the age groups of 0-15 years and above 75 years respectively.

The maximum numbers of *P. aeruginosa* and *A. baumannii* isolates were obtained from samples such as pus 30% (n=26) followed by blood 20% (n=18), urine 17% (n=15), ear swab 18% (n=16), tissues 8% (n=7) and sputum 7% (n=6). The distribution of various samples that yielded *Pseudomonas* and *Acinetobacter* isolates, is discussed in figure 2, with pus samples and blood samples representing a significant proportion of *P. aeruginosa* and *A. baumannii* respectively. Tissue samples showed the least amount of isolation. Moreover, no *Acinetobacter spp* was isolated from ear swabs and sputum samples.

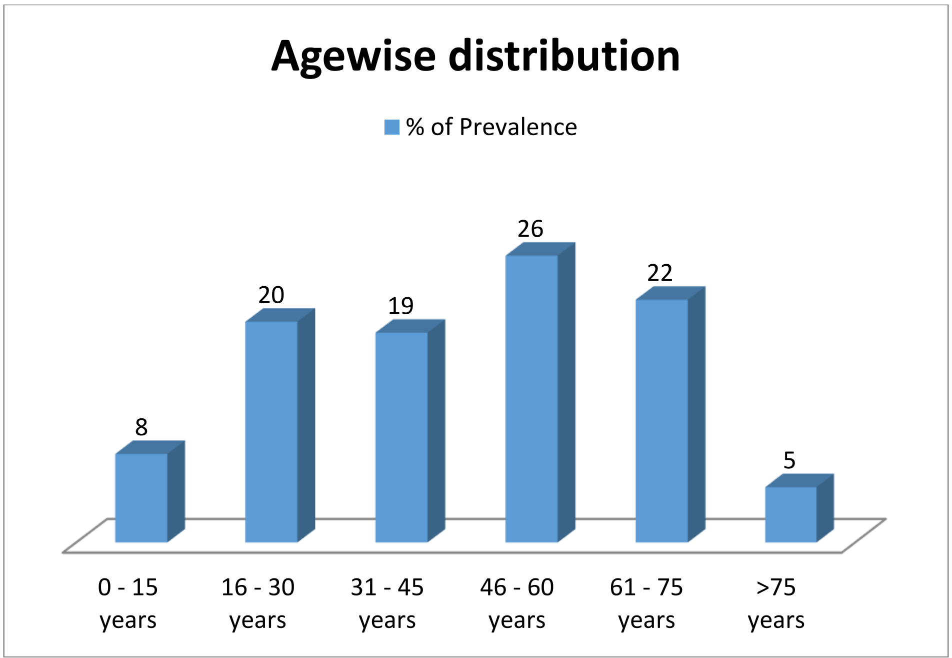

Figure 1: Age-wise distribution of Pseudomonas and Acinetobacter species

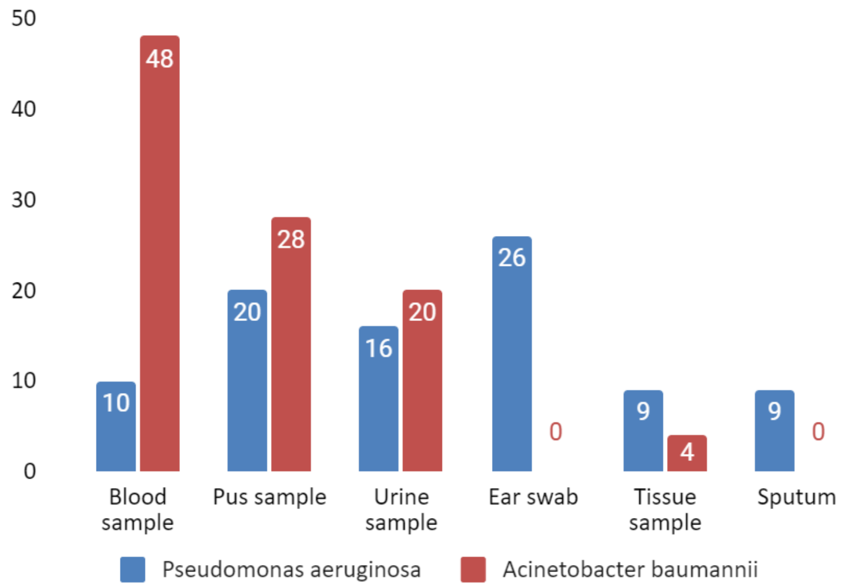

Figure 2: Distribution of *P. aeruginosa* and *A. baumannii* among various clinical samples

Table 1
Specimen-wise distribution of antibiotic resistance in *A. baumannii*

Antibiotics	Blood (n =12)	Tissue (n =1)	Pus (n =7)	Urine (n =5)
Ceftazidime	1	1	7	2
Cefepime	1	1	7	0
Piperacillin/Tazobactam	2	0	7	2
Ciprofloxacin	3	1	7	2
Levofloxacin	2	1	7	0
Amikacin	3	0	7	1
Gentamicin	3	0	7	3
Tobramycin	0	0	0	0
Co-trimoxazole	3	1	7	2
Imipenem	5	1	5	2
Meropenem	1	0	4	0
Colistin	3	0	4	1
Polymyxin B	0	0	0	0

Table 2
Specimen-wise distribution of antibiotic resistance in *P. aeruginosa*

Antibiotics	Blood (n =6)	Sputum (n = 6)	Tissue (n =6)	Pus (n =19)	Ear swab (n =16)	Urine (n =10)
Ceftazidime	0	0	1	0	0	4
Cefepime	0	0	1	0	0	3
Piperacillin/Tazobactam	0	0	1	0	1	2
Ciprofloxacin	0	1	1	1	5	6
Levofloxacin	0	0	1	3	3	2
Amikacin	0	0	0	2	2	3
Gentamicin	0	1	1	7	3	4
Tobramycin	0	0	1	1	2	4
Intrinsic resistance to Co-trimoxazole						
Imipenem	0	0	0	0	1	4
Meropenem	0	2	1	0	1	4
Colistin	0	0	0	0	0	0
Polymyxin B	0	0	0	0	0	0

The prevalence of MDR phenotype was high in *A. baumannii* (60%) compared to *P. aeruginosa* isolates (14%) in this study. The specimen-wise distribution of the antimicrobial resistance pattern of *A. baumannii* and *P. aeruginosa* is shown in tables 1 and 2. Among *A. baumannii* isolates, the highest resistance (52%) was observed for ciprofloxacin, gentamicin, co-trimoxazole and imipenem. The second highest resistance was ceftazidime, piperacillin/tazobactam and amikacin. All *A. baumannii* isolates were susceptible to tobramycin and polymyxin B. Resistance to colistin was seen in 32% of the isolates. Among isolates of *P. aeruginosa*, 25.3% were resistant to gentamicin, 22.2% to ciprofloxacin and less than 10% to other tested antibiotics. All *P. aeruginosa* isolates were susceptible to colistin. Polymyxin B. *Pseudomonas spp* is intrinsically resistant to co-trimoxazole.

Discussion

It is of great concern when MDR isolates of Pseudomonas spp. and Acinetobacter spp. are acquired nosocomial. Antibiotic selection for empirical treatment is challenging and mainly relies on institutional-level susceptibility data. Unfortunately, lack of prescription guidelines, restricted availability of antimicrobial drugs and inadequate standard microbiology laboratory tests to support antibiotic selection, all contribute to the complexity of the situation. Further, the impact of MDR infections on patient outcomes remains to be investigated, although these resistant pathogens are a recognized problem in Asia including India.

In our study, the prevalence of *P. aeruginosa* was 74% while 26% of patients were infected with *A. baumannii*. This percentage of isolation was similar to the study conducted by Grewal et al[7] where 88% of *P. aeruginosa* and 8% of *A. baumannii* were isolated from various clinical samples. The variation in these percentages could be due to the varying prevalence rates of NFGNB in different hospital settings and geographical areas. *P. aeruginosa* was the predominant pathogen in both studies. Furthermore, most of *A. baumannii* isolates were of blood origin followed by pus while *P. aeruginosa* isolates from ear swabs were followed by pus samples.

However, other studies from India showed ET (Endotracheal) secretion as the major source[11,12]. On the other hand, Kaur et al[9] had shown urinary isolates to be the most common, contrary to the present study.

The incidence of NFGNB was high among males (64%) compared to females (36.6%). The predominance of male patients infected with NFGNB has also been shown in other studies[2,6,14]. The increasing number of men reporting to hospitals might be the cause of this observation. The maximum number of isolates were recovered from the 46-60 years age group which is consistent with the previous studies. The rate of positivity was high among the 45-64 years and with a similar age group of 42-68 years by Uwingabaye et al[15]. These increased rates would be associated with lower immune status such as old age, immunosuppression and patients on chemotherapy and steroids.

Infections due to MDR *A. baumannii* and *P. aeruginosa* were observed in 60% and 14% of the study isolates which are less compared to other studies. This could be due to the variations in the number of samples studied. These isolates are believed to cause greater mortality, longer hospital stays and higher healthcare expenses compared to susceptible bacteria [16]. A study by Uwingabiye et al[15] revealed a higher percentage (92.6%) of MDR *Acinetobacter* in ICUs which is in line with the current study. Worldwide recognition of fluoroquinolone resistance in NFGNB has been growing recently which was also observed in the present study.

Carbapenems are one of the most effective drugs against NFGNB, however, resistant strains have arisen. The overall resistance to carbapenems was less (<20%) comparable to that of studies reported from India (34%)[16]. Drugs like tigecycline and colistin have demonstrated efficacy against the majority of MDR strains of *A. baumannii*. In this study, 32% of *Acinetobacter* isolates showed colistin resistance however, colistin resistance among *Pseudomonas* isolates was not identified. Notably, *A. baumannii* and *P. aeruginosa* were susceptible to polymyxin B. Very few studies support this drug's effectiveness against this organism, although it is frequently not recommended in clinical practice due to its nephrotoxicity and neurotoxicity[16]. However, more research in clinical practice is required to evaluate this. On the other hand, pan-resistant *A. baumannii* was not isolated.

As baseline data on the local dissemination of MDR isolates of Pseudomonas and Acinetobacter species, the current study may help to raise awareness of the issue among doctors, other healthcare professionals, researchers and public health regulators. Strict infection prevention and control measures together with antibiotic stewardship initiatives, should be put into place in ICU units where resistant strains are spreading.

Conclusion

In conclusion, our study highlights the high prevalence of NFGNB in ICUs at our tertiary care hospital in South India. The study documents the carbapenem and colistin-resistant strains as these strains have fewer treatment options and increase the risk of treatment failure. It is important to monitor and regulate the use of antibiotics in healthcare settings to help prevent the emergence of antimicrobial-resistant strains of bacteria. Furthermore, the need for regular surveillance and monitoring of these bacteria are vital to understand better and to respond to the resistance patterns in the hospital population.

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