

## Short Communication

# Prevalence and antimicrobial susceptibility of non-fermenting Gram-negative bacilli isolated from clinical samples at a tertiary care hospital

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### Abstract

**Introduction:** We compared the prevalence and antimicrobial susceptibility of non-fermenting gram-negative bacilli (NFGNB) isolated from clinical samples at a Brazilian tertiary care hospital in 2008 and 2013. **Methods:** Collected data included patient's name, age, sex, inpatient unit, laboratory record number, type of biological material, culture test result, and antimicrobial susceptibility of isolated strains. **Results:** Out of 19,112 culture tests analyzed, 926 (4.8%) were positive for NFGNB. Among these, 45.2% were metallo-beta-lactamase (MBL) producing strains. **Conclusion:** Between 2008 and 2013, the number of MBL-producing NFGNB isolates increased by 21.5%, which was accompanied by a consequent reduction in susceptibility to antimicrobials.

**Keywords:** Bacterial resistance. Metallo-beta-lactamase. Nosocomial infections.

Non-fermenting, gram-negative bacteria (NFGNB) are described as a strictly aerobic and non-sporulating group of microorganisms that rely on oxidative pathways because they are unable to get energy from carbohydrates by fermentation<sup>1</sup>. They are opportunistic bacteria with a low level of virulence that seldom cause disease in healthy individuals. However, they may cause severe infections in hospitalized, immunocompromised, and intensive care unit (ICU) patients. NFGNB do not require many nutrients for their development, can tolerate harsh environmental conditions, show remarkable resistance to antimicrobials, and are frequently described as hospital-acquired opportunistic pathogens<sup>2-4</sup>. Multidrug resistance of NFGNB stems from different factors, such as up-regulated production of enzymes metabolizing the drugs, target site changes, overexpression of efflux pumps, and porin deficiency<sup>5,6</sup>. Metallo-beta-lactamase (MBL) production, mainly by *Pseudomonas aeruginosa*, stands out as a frequent cause of severe nosocomial infections<sup>7,8</sup>.

The prevalence, phenotypic characteristics, and antimicrobial susceptibility profile of NFGNB strains may show regional variation. Therefore, epidemiological studies are needed to establish appropriate therapeutic management strategies to prevent the infections caused by NFGNB.

Accordingly, we conducted a descriptive, observational, and cross-sectional study and evaluated the prevalence and antimicrobial susceptibility profile of NFGNB strains in isolates from patients admitted to Hospital São Vicente de Paulo (HSVP), Passo Fundo, State of Rio Grande do Sul, Brazil. These results were compared with a similar set of data collected from the same hospital in 2008 to assess the development of antimicrobial resistance in recent years.

The patients' samples were collected for bacterial culture tests during January to December, 2013. All data regarding bacterial culture tests performed during 2013 were collected from the HSVP computer system using data management software available at the Clinical Laboratory. The following data were collected: patient's name, age, sex, inpatient unit, patient's laboratory record number, biological material type, culture test result, and drug susceptibility of isolated strains. Data from patients whose culture tests were positive for NFGNB were analyzed further. Bacterial isolates were identified by classic biochemical and morphological tests. Antimicrobial susceptibility was assessed by the disk diffusion method, according to Clinical Laboratory Standards Institute guidelines<sup>9</sup>. The antimicrobials tested were as follows: amikacin (30µg), ampicillin/sulbactam (20µg), aztreonam (30µg), cefepime (30µg), ceftazidime (30µg), ceftriaxone (30µg), ciprofloxacin (5µg), gentamicin (10µg), meropenem (10µg), piperacillin/tazobactam (110µg), and tobramycin (10µg). Isolates that were not sensitive to the tested antimicrobials were considered resistant.

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Frequencies of both positive culture tests and non-fermenting gram-negative bacilli isolation were calculated. After that, the percentage of MBL-producing and non-producing (MBL and non-MBL) strains was determined. These data were compared with the information about the type of biological sample, susceptibility to the tested antimicrobials, and inpatient unit, as well as analyzed with respect to patients' sex and age, using the  $\chi^2$  test implemented in the Statistical Package for the Social Science (SPSS), version 22. The significance level was set at 5%.

Out of 19,112 cultures analyzed, 926 (4.8%) were positive for NFGNB. Among those, 51.9% (481/926) were represented by *P. aeruginosa*, 35.6% (330/926) - by *Acinetobacter baumannii*, and 12.4% (115/926) - by *Pseudomonas* sp. (but not *Pseudomonas aeruginosa*).

NFGNB were sorted based on the presence of MBL. This enzyme was produced by 74.8% (247/330) of *A. baumannii*, 30.1% (145/481) of *P. aeruginosa*, and 23.5% (27/115) of *Pseudomonas* sp. isolates. Overall, 45.2% of strains (419/926) produced MBL, whereas 54.8% (507/926) did not.

**Table 1** displays the frequencies of MBL and non-MBL *A. baumannii*, *P. aeruginosa*, and *Pseudomonas* sp. isolates, depending on the type of biological sample, inpatient unit, and patients' sex and age. *A. baumannii* samples were obtained mainly from tracheal aspirate (MBL: 55.9%, 138/247; non-MBL: 50.6%, 42/83) and that prevalence was statistically significant. MBL-producing *P. aeruginosa* strains were mostly obtained from urine samples (36.5%; 53/145), whereas non-MBL strains were found mainly in other biological materials,

TABLE 1

Relative proportions of *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Pseudomonas* sp. isolates that produced or did not produce MBL in isolate groups classified by the type of biological sample, inpatient unit, gender, and age of patients (%).

	<i>Acinetobacter baumannii</i> (n = 330)		<i>Pseudomonas aeruginosa</i> (n = 481)		<i>Pseudomonas</i> sp. (n = 115)	
	MBL (n = 247)	non-MBL (n = 83)	MBL (n = 145)	non-MBL (n = 336)	MBL (n = 27)	non-MBL (n = 88)
<b>Biological sample</b>						
urine	2.0	0	36.5	11.6	22.2	13.6
sputum	12.1	13.3	8.3	10.4	3.7	13.6
tracheal aspirate	55.9	50.6	22.8	25.0	22.2	18.2
body fluids*	30.0	36.1	32.4	53.0	51.9	54.6
<b>Inpatient unit</b>						
central ICU	52.2	37.4	22.1	16.7	22.2	8.0
pediatric ICU	2.0	8.4	2.1	8.9	7.4	3.4
neonatal ICU	0	2.4	0.7	1.5	0	0
cardiology ICU	0.8	2.4	0.7	1.8	0	6.8
general wards**	45.0	49.4	74.4	71.1	70.4	81.8
<b>Gender</b>						
male	61.1	69.9	58.6	61.6	51.8	52.3
female	38.9	30.1	41.4	38.4	48.2	47.7
<b>Age (years)</b>						
0–2	1.2	9.6	2.1	8.9	0	6.8
3–12	2.4	3.6	1.4	7.5	7.4	5.7
13–25	3.2	3.6	10.3	7.7	3.7	6.8
26–45	16.2	16.9	12.4	14.0	7.4	9.1
46–60	22.7	22.9	24.1	11.9	11.1	19.3
>60	54.3	43.4	49.7	50.0	70.4	52.3

MBL: metallo-beta-lactamases; ICU: intensive care unit. \*Body fluids: blood, stools, bronchoalveolar lavage, catheter tip, and wound secretion. \*\*General wards: hospital clinics, emergency rooms, operating theater, and recovery room.

including blood, feces, bronchoalveolar lavage, catheter tip, and surgical wound infections (53%; 178/336). Predominance of MBL and non-MBL *P. aeruginosa* strains in respective samples was statistically significant. Both MBL and non-MBL *Pseudomonas* sp. strains were mostly isolated from other biological materials as well (MBL: 51.9%, 14/27; non-MBL: 54.6%, 48/88), and that prevalence was also statistically significant. With regard to inpatient units, most of MBL *A. baumannii* strains were obtained from the Central ICU (52.2%; 129/247), whereas non-MBL strains were mainly collected from general wards, such as emergency room, recovery room, surgical ward, and hospital admission premises (49.4%; 41/83). Both MBL and non-MBL *P. aeruginosa* and *Pseudomonas* sp. strains were also mostly obtained from general wards. All results were statistically significant. With respect to age, high prevalence of the three bacteria was noted among patients older than 60 years, whereas their presence in samples from those younger than 25 years was rare ( $P < 0.001$ ).

The antimicrobial resistance of MBL and non-MBL strains of the three bacteria was then assessed. MBL *A. baumannii* strains were highly resistant to most of the tested antibiotics; all strains exhibited reduced susceptibility to cefepime, ceftriaxone, and meropenem, and over 99% of the strains were resistant to ciprofloxacin, piperacillin/tazobactam, and ceftazidime. Amikacin turned out to be the best treatment option against *A. baumannii*, as only 34% of *A. baumannii* strains were resistant to it (Figure 1). Over 50% of non-MBL *A. baumannii* strains had reduced susceptibility to all tested cephalosporins (78.3% - to ceftriaxone, 57.8% - to ceftazidime, and 55% - to cefepime) and also to ciprofloxacin (50.6%). For all other tested antimicrobials, reduced susceptibility was noted in less than 50% of the strains. MBL *P. aeruginosa* strains also showed high resistance to most of the tested antimicrobials: all strains were resistant to meropenem, 93.1% - to ciprofloxacin, and 85.5% - to gentamicin. Aztreonam, to which only 23.4% of *P. aeruginosa* strains were resistant, was the best option in this case (Figure 2). In contrast, non-MBL *P. aeruginosa* strains showed much lower resistance to antimicrobials. Most frequently, reduced susceptibility was noted in the case of amikacin (22.8%), followed by the resistance to gentamicin (17.6%) and ciprofloxacin (17%). MBL-producing *Pseudomonas* sp. strains were highly resistant to meropenem (100%), ceftazidime (92.6%), and aztreonam (85.2%). Amikacin was the best treatment option against *Pseudomonas* sp., as only 37% of the strains were resistant to it. Non-MBL *Pseudomonas* sp. strains revealed higher susceptibility to antimicrobials, and the highest rate of resistance was noted in the case of aztreonam (25%).

With regard to the frequency of NFGNB (4.8%), in a study of samples isolated between June and December of 2008 in the same hospital, Machado *et al.* found a lower occurrence of positive cultures (2.8%; 223/7,849)<sup>10</sup>. In that study, *P. aeruginosa* was the most frequent species (77.6%; 173/223), followed by *Acinetobacter* sp. (22.4%; 50/223). Deliberati *et al.* found that NFGNB comprised 2.2% of all strains isolated between 2006 and 2008 from a hospital in Porto Alegre, Rio Grande do Sul, Brazil<sup>11</sup>. *P. aeruginosa* was also the

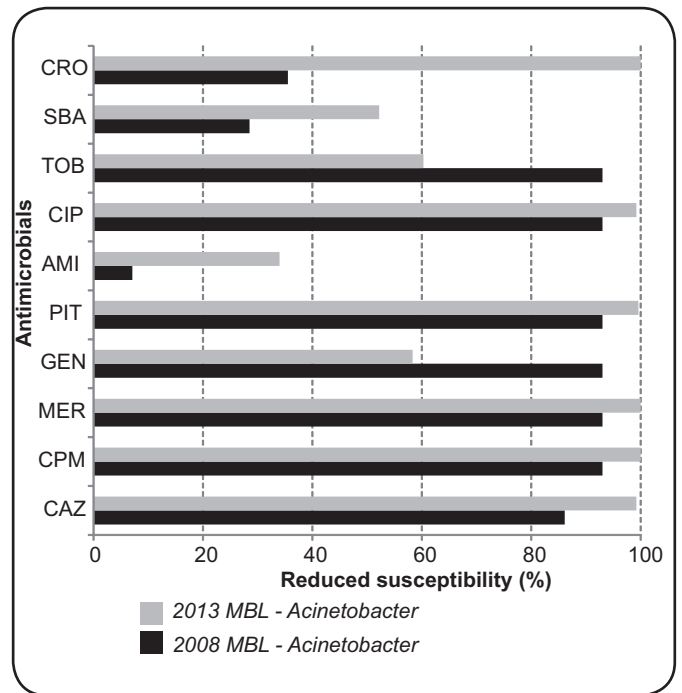


FIGURE 1 - Percentage of antimicrobial-resistant MBL producing *Acinetobacter* sp. strains isolated in 2008 and 2013. CRO: ceftriaxone; SBA: ampicillin/sulbactam; TOB: tobramycin; CIP: ciprofloxacin; AMI: amikacin; PIT: piperacillin/tazobactam; GEN: gentamicin; MER: meropenem; CPM: ceftazidime; CAZ: ceftazidime; MBL: metallo-beta-lactamase.

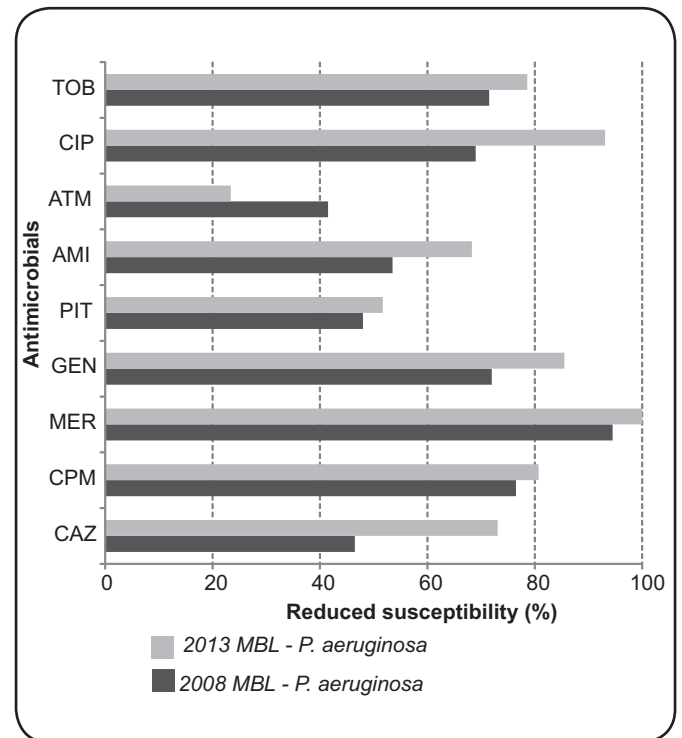


FIGURE 2 - Percentage of antimicrobial-resistant MBL producing *Pseudomonas aeruginosa* strains isolated in 2008 and 2013. TOB: tobramycin; CIP: ciprofloxacin; ATM: aztreonam; AMI: amikacin; PIT: piperacillin/tazobactam; GEN: gentamicin; MER: meropenem; CPM: ceftazidime; CAZ: ceftazidime; MBL: metallo-beta-lactamase; *P.*: *Pseudomonas*.

predominant species (65%), followed by *A. baumannii* (16.5%). That frequency of *A. baumannii* strains was considered high by the authors, who commented that *A. baumannii* was an emerging hospital pathogen. In our study, *A. baumannii* strains were isolated even more frequently (35.6%), which confirms the growing importance of this species in the hospital environment. Juyal *et al.* reported a higher rate of NFGNB (9.3%) in India<sup>12</sup>. In another part of India, Benachinmardi *et al.* detected NFGNB at a much lower rate of 3.6%<sup>13</sup>, which was similar to the rate observed herein.

Furthermore, by comparing the data obtained in this study with those reported by Machado *et al.* for the same hospital<sup>10</sup>, we noted that the fraction of MBL producing strains rose from 23.7% to 45.2% within five years (between 2008 and 2013). In 2008, 17.4% of *P. aeruginosa* isolates and 6.3% of *Acinetobacter* sp. strains tested positive for MBL production. In 2013, these rates went up to 74.8%, and 30.1% for *A. baumannii* and *P. aeruginosa*, respectively. High initial numbers and large relative increase in the occurrence of MBL-producing *A. baumannii*, compared to 2008 data, are particularly notable.

With regard to the relationship between NFGNB occurrence and patient age, we observed that NFGNB were prevalent among individuals aged over 65 years, indicating that comorbidities that develop with age likely influence the invasiveness of NFGNB. Machado *et al.* also found higher prevalence of *P. aeruginosa* and *Acinetobacter* sp. among individuals over 60 years old<sup>10</sup>.

As in the study by Machado *et al.*<sup>10</sup>, MBL strains were mainly isolated from tracheal aspirate (42.2%; 177/419). These samples are usually collected from mechanically ventilated patients, and in such cases, the airways are often colonized by NFGNB, especially *P. aeruginosa*<sup>13</sup>. Similar observations were reported by Deliberali *et al.*, where most NFGNB strains were isolated from tracheal aspirate (38.3%)<sup>11</sup>.

The Figures compare the proportions of antimicrobial-resistant MBL-producing *Acinetobacter* (**Figure 1**) and *P. aeruginosa* (**Figure 2**) strains isolated in 2008 and 2013. By examining these profiles, we noted that although resistance to amikacin increased from 7.1% (in 2008) to 34% (in 2013), it remained the most effective antimicrobial against infections caused by MBL-producing *Acinetobacter* strains. Ampicillin/sulbactam was second most potent drug, but the resistance to it also increased from 28.5% in 2008 to 52.2% in 2013. We also observed that gentamicin and tobramycin were the only antimicrobials, to which *Acinetobacter* strains became more sensitive between 2008 and 2013. The resistance of *Acinetobacter* strains to all other tested antimicrobials increased in that period. The increase in the resistance of these strains to ceftriaxone was noteworthy: from 35% in 2008 to 100% in 2013. Compared to susceptibility parameters in 2008, MBL *P. aeruginosa* strains also showed higher rates of resistance to all tested antibiotics except aztreonam in 2013.

Our results indicate high levels of resistance of NFGNB (mainly MBL-producing) to multiple tested antimicrobials. Moreover, infections were more prevalent in hospital wards with a large number of immunocompromised and older patients. The increase in resistance can be explained, among other factors,

by the change in the profile of patients admitted to HSVP. In the last years, HSVP started treating patients with more severe disorders, which led to an eventual increase in the number of invasive procedures and the use of antimicrobials, especially carbapenem antibiotics. The adoption of continued education measures, mainly with the aim of raising awareness about the importance of hand hygiene, is crucial for limiting the increase in antibiotic resistance. Furthermore, the implementation of policies for the control and restriction of antimicrobial use and for isolation of infected or colonized patients is of paramount importance, as higher resistance limits antibiotic treatment options.

Regional and chronological changes in the antimicrobial susceptibility of bacteria highlight the importance of epidemiological surveys in hospitals. The development of therapeutic protocols for tuning antibiotic therapy regimens is also important to minimize the dissemination of these antibiotic-resistant bacterial strains and other pathogens.

### Ethical considerations

The experiments were performed in accordance with the Brazilian National Council of Research Ethics (CONEP) Resolution 466/12 and the Declaration of Helsinki. The study was approved by the Research Ethics Committee of the *Universidade de Passo Fundo*, affiliated with the CONEP, under number 768.158.

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### Conflict of interest

The authors declare that there is no conflict of interest.

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