

## Original Research Article

# Prevalence and antibiogram of nonfermenting gram negative bacilli isolates obtained from various clinical samples in a tertiary care hospital, Bathinda, Punjab, India

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### ABSTRACT

**Background:** Non-fermenting gram-negative bacilli (NFGNB) have emerged as important healthcare associated pathogens in recent years. Infections caused by these bacteria are almost always secondary to some predisposing factors in patients such as burns, prolonged antimicrobial therapy, immunosuppression etc. The Objectives of the study was to be carried out with an objective to identify NFGNB upto genus and species level and study their antimicrobial sensitivity/ resistance pattern so that empiric therapy could be selected accordingly.

**Methods:** A total of 2261 clinical samples were collected from patients admitted in ICU and different wards of the hospital. All samples were processed according to standard microbiological procedures. Identification of NFGNB upto genus and species level was done by various biochemical tests. Antimicrobial susceptibility testing was done by Kirby Bauer disc diffusion method results were interpreted in accordance with clinical laboratory standards institute guidelines.

**Results:** In this study, 365 NFGNB were obtained accounting for their prevalence of 16.1%. *P. aeruginosa* was the commonest NFGNB isolated in this study accounting for 52.6%, *A. baumannii* was the second common NFGNB isolated (31.7%). Other NFGNB isolates were obtained with a lesser frequency. *P. aeruginosa* isolates were highly sensitive to polymyxin B and colistin followed by imipenem. Most of the *A. baumannii* isolates were multidrug resistant.

**Conclusions:** This study gives an alarming sign towards high prevalence of multi drug resistant NFGNB in our hospital. Therefore, improved antibiotic stewardship and strict protocols for hand washing need to be implemented to prevent emergence and spread of multidrug resistant NFGNB in health care settings.

**Keywords:** *A. baumannii*, Antibiotic resistance, Non-fermenting gram-negative bacilli, *P. aeruginosa*

### INTRODUCTION

The non-fermentative gram negative bacilli (NFGNB) consists of a diverse group of non-spore forming, aerobic bacilli that either do not use carbohydrates as the source of energy or degrade them through metabolic pathways other than fermentation.<sup>1</sup> Certain conditions or diseases

predispose the patients to infection with non-fermenters like malignancies particularly of reticuloendothelial system, instrumentation, surgery, catheterizations particularly of urinary tract, intravascular catheterisation, lumbar puncture, tracheostomy, dialysis, lavages, placement of shunts, prosthesis and prolonged antibiotic usage and chronic infections. Burns, open wounds and

exudative lesions are other predisposing factors.<sup>2</sup> NFGNB account for about 15% of all gram-negative bacilli isolated from clinical specimens. Infections caused by these bacteria are almost always secondary to some predisposing factors in patients such as burns, prolonged antimicrobial therapy, introduction of immunosuppressive agents, extremes of age and prolongation of life of a patient with many severe diseases, by advanced surgical and medical treatment.<sup>3</sup> In routine, they are identified only in few laboratories in India as they are slow growing and require special culture media and biochemical tests for their identification.<sup>4-6</sup> Resistance to antimicrobials is common in these organisms and has increased over the years among NFGNB and number of strains are now resistant nearly to all commonly used antibiotics. Development of resistance in non-fermenters is multifactorial. Factors involved are mutations in genes encoding porins, efflux pump mechanisms, penicillin binding proteins, chromosomal beta lactamases.<sup>7</sup> Success of antimicrobial therapy depends on the appropriateness of the choice of antibiotics that should be used on the basis of prior knowledge of the susceptibility pattern of the agent; therefore this study was conducted with an objective to identify non-fermenting Gram negative bacilli isolated from various clinical samples upto genus and species level along with study of their antimicrobial sensitivity/resistance pattern.

## METHODS

This present study was conducted in Bacteriology Section of Microbiology Department, Adesh Institute of Medical Sciences and Research (AIMSR), Bathinda after getting approval from the Thesis Research Degree Committee and Ethical Committee of the Institute.

A total of 2261 clinical samples were collected from patients admitted in ICU and various wards of the hospital of depending upon the clinical diagnosis of respective patients. These included: urine, pus, blood, ear swabs, high vaginal swabs, sputum, endotracheal secretions, tracheal aspirate and various body fluids. Out of total samples, 858 samples were collected from ICU patients and 1403 samples were collected from patients admitted in various wards of the hospital.

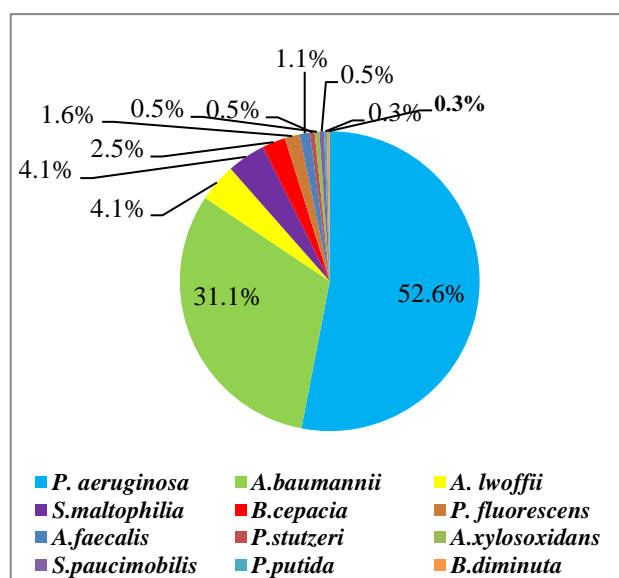
All samples were collected and processed as per standard microbiological guidelines. Samples were inoculated on to Blood Agar (BA) and MacConkey Agar (MA) plates under strict aseptic conditions and plates were incubated at 37°C for 24-48 hours under aerobic conditions. All isolates that showed non-lactose fermenting colonies on MA and those which grew only on BA and not on MA were subjected to Gram staining and all gram-negative bacilli/cocci/coccobacilli obtained were then subjected to triple sugar iron test. The bacterial isolates which produced alkaline/acid (K/A) reaction and acid/acid (A/A) reaction were excluded. Isolates which produced an alkaline/alkaline (K/K) reaction were provisionally

identified as non-fermenters and were included in this study and subjected to identification upto genus/species level by a battery of biochemical tests.<sup>8</sup> Oxidative/Fermentative (O/F) test for glucose, lactose, sucrose, mannitol and xylose, oxidase test, motility test, nitrate reduction test, lysine and ornithine decarboxylase test, arginine dihydrolase test, gelatin liquefaction test, urease test, indole production test, citrate utilization test, growth at 42°C and 44°C. Antimicrobial susceptibility testing was performed by Kirby Bauer disc diffusion method on Mueller Hinton agar as per CLSI guidelines using commercially available discs.<sup>9,10</sup> Following antimicrobial discs were used: ceftazidime (30µg), cefepime (30µg), piperacillin-tazobactam (100µg/10 µg), aztreonam(30µg), imipenem (10µg), meropenem(10 µg), gentamicin (10µg), amikacin (30µg) netilmicin (30 µg), ciprofloxacin (5µg), norfloxacin (30µg; for urinary isolates), polymyxin B (300 units) and colistin (10µg). Plates were incubated at 37° C for 18-24 hours and results were interpreted according to zone sizes mentioned in the CLSI guidelines.<sup>10</sup>

*P. aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 25922 were used as control strains. All dehydrated media and antibiotic discs were procured from HiMedia Labs, Mumbai, India. Statistical analysis was done by descriptive statistics using percentages and ratios method.

## RESULTS

Out of total samples processed, 1136 (50.3%) samples showed growth and in 1125 (49.7%) samples, no growth was obtained after appropriate incubation period and a total of 365 NFGNB were isolated thus accounting for their isolation rate as 16.1%. 270 (76%) isolates were obtained from male patients and 95 (24%) were isolated from female patients.



**Figure 1: Distribution of various NFGNB isolates obtained in the study.**

Maximum NFGNB (37.4%) were obtained from age group of 41 to 60 years followed by age- group of 21 to 40 years; 61-80 years; 0 to 20 years and minimum isolates were obtained from age group of more than 80 years. 208 NFGNB isolates were obtained from ICU patients and 157 were isolated from patients admitted in different wards. *P. aeruginosa* was the commonest NFGNB isolated in this study with the prevalence of 52.6% among total NFGNB isolates. *A. baumannii* was the second common NFGNB isolated (31.7%) and others accounted for a total of 15.6%. These included *P. fluorescens*, *P. stutzeri*, *P. putida*, *A. lwoffii*, *S. maltophilia*, *A. faecalis*, *S. paucimobilis*, *B. cepacia*, *A. xylosoxidans* and *B. diminuta* (Figure 1).

Maximum NFGNB isolates were obtained from pus samples followed by urine, endotracheal secretions and tracheal aspirate. Sputum and blood, each accounted for 4.9% and 3.6% of total isolates were obtained from ear swabs. Very few isolates were obtained from CSF, intercostal fluid, pleural fluid and central line tip culture as shown in Figure 2. Sample-wise distribution of all NFGNB isolates obtained is shown in Table 1.

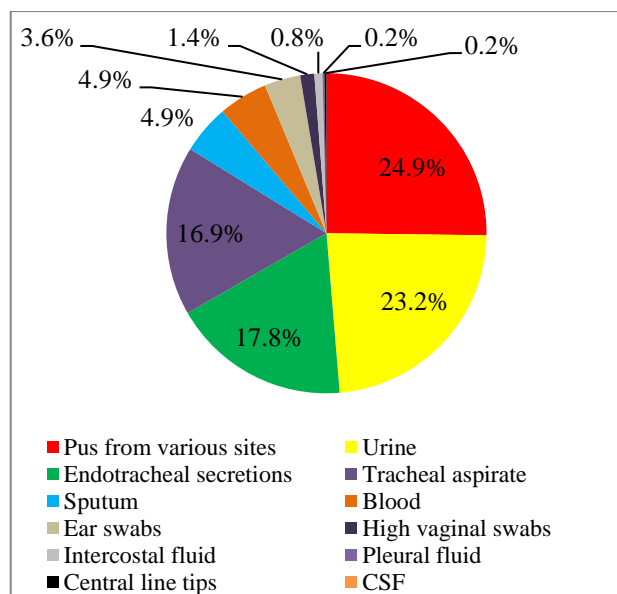


Figure 2: Distribution of various NFGNB isolates obtained from various samples.

Table 1: Sample-wise distribution of various NFGNB isolates.

Type of sample	Total isolates	<i>P. aeruginosa</i>	<i>P. fluorescens</i>	<i>P. stutzeri</i>	<i>P. putida</i>	<i>A. baumannii</i>	<i>A. lwoffii</i>	<i>S. maltophilia</i>	<i>B. cepacia</i>	<i>A. faecalis</i>	<i>B. diminuta</i>	<i>A. xylosoxidans</i>	<i>S. paucimobilis</i>
Urine	85	54	5	1	1	9	5	1	2	4	1	2	1
Pus	91	54	-	1	-	29	5	1	-	-	-	-	-
Blood	18	41	-	-	-	6	1	3	4	-	-	-	-
Tracheal aspirate	62	29	-	-	-	28	1	3	1	-	-	-	-
Et secretions	65	20	-	-	-	34	3	6	2	-	-	-	-
Sputum	18	10	-	-	-	7	-	1	-	-	-	-	-
HVS	5	4	-	-	-	-	-	-	-	-	-	-	1
Ear swabs	14	13	1	-	-	-	-	-	-	-	-	-	-
Pleural fluid	2	2	-	-	-	-	-	-	-	-	-	-	-
Intercostal fluid	3	1	-	-	-	2	-	-	-	-	-	-	-
CSF	1	1	-	-	-	1	-	-	-	-	-	-	-
Central line tips	1	-	-	-	-	-	-	-	-	-	-	-	-
Total	365	192	6	2	1	116	15	15	9	4	1	2	2

*P. aeruginosa* isolates were 100% sensitive to polymyxin B and colistin, 71.4% sensitivity was reported towards imipenem and very less sensitivity was reported towards cephalosporins. *A. baumannii* isolates showed high level of resistance to most of the antibiotics tested. However, 96.5 % sensitivity was recorded for polymyxin B and 97.4% for colistin indicating them as the drugs to be used for treatment of *A. baumannii* infections. Antibiogram obtained for *A. lwoffii* was observed as much more

susceptible to antibiotics tested. *B. cepacia* showed very good sensitivity towards cefepime (77.7%), imipenem (66.6%), meropenem (77.7%) and all isolates were sensitive to piperacillin-tazobactam and cotrimoxazole. *S. maltophilia* was also found to be multidrug resistant pathogen showing resistance to various groups of antibiotics. All isolates of *S. maltophilia* were resistant towards ceftazidime, cefepime, imipenem and meropenem. All isolates were sensitive to cotrimoxazole.

Antibiotic susceptibility pattern obtained for most commonly isolated NFGNB is shown in Table 2.

**Table 2: Antibiotic susceptibility profile of commonly isolated NFGNB in present study.**

Antibiotic Tested	<i>P. aeruginosa</i> (n=192)	<i>A. baumannii</i> (n=116)	<i>A. lwoffii</i> (n=15)	<i>B. cepacia</i> (n=9)	<i>S. maltophilia</i> (n=15)
Ceftazidime	75 (39.1%)	4 (3.4%)	8 (53.3%)	5 (55.5%)	0 (0%)
Cefepime	81 (42.2%)	6 (5.2%)	9 (60%)	7 (77.7%)	0 (0%)
Piperacillin-tazobactam	124 (64.6%)	15 (12.9%)	13 (86.6%)	9 (100%)	4 (26.6%)
Cotrimoxazole	Not tested	9(7.8%)	10 (66.6%)	9 (100%)	15 (100%)
Aztreonam	86 (44.8%)	Not-tested	Not tested	Not tested	Not tested
Gentamicin	107 (55.7%)	11 (9.5%)	14 (93.3%)	1 (11.1%)	2 (13.3%)
Amikacin	117 (60.9%)	12 (10.3%)	12 (80%)	0 (0%)	2 (13.3%)
Netilmicin	120 (62.5%)	Not tested	Not tested	Not tested	Not tested
Ampicillin-sulbactam	Not tested	30 (25.8%)	14 (93.3%)	Not tested	Not tested
Norfloxacin (for urinary isolates)	26 (48.1%)	2 (22.2%)	3 (60%)	0 (0%)	Not tested
Ciprofloxacin (for non-urinary isolates)	79 (57.2%)	12 (11.3%)	7 (70%)	2 (28.5%)	8 (53.3%)
Imipenem	137 (71.4%)	46 (39.6%)	15 (93.3%)	6 (66.6%)	0 (0%)
Meropenem	124 (64.5%)	37 (31.9%)	15 (93.3%)	7 (77.7%)	0 (0%)
Polymyxin b	192 (100%)	112 (96.5%)	15 (100%)	0 (0%)	15 (100%)
Colistin	192 (100%)	113 (97.4%)	15 (100%)	0 (0%)	15 (100%)

## DISCUSSION

Non-fermenters were usually considered as commensals or contaminants in the past but have now emerged as important health care pathogens. These organisms are associated with life threatening infections such as septicemia, pneumonia, UTI, meningitis, surgical site infections, ventilator associated pneumonia, osteomyelitis etc. and resistance to antimicrobials have resulted in difficulty in treatment of infections caused by these bacteria.<sup>11</sup> NFGNB are intrinsically resistant to various antimicrobials and are known to produce extended spectrum betalactamases (ESBL's) and metallobetalactamases (MBL's).<sup>12</sup> In the present study, isolation rate of NFGNB out of total samples processed was 16.1%. Similar isolation rate has been reported by other authors: 16%, 19%, 12.2%.<sup>7,13,14</sup> Very less isolation rate has been reported by Benanchinmardi et al and Malini et al i.e. 3.5% and 4.5% respectively.<sup>6,15</sup> However, some authors have also reported higher isolation rates of NFGNB in their studies: 75.9%, 66.8% and 36.5%.<sup>16-18</sup> 76% isolates of NFGNB were obtained from male patients and 24% from female patients. These results are similar to Jayapriya et al who has reported NFGNB isolates from males as 71% and females as 29%.<sup>19</sup> In another study by Ridhima et al 69.7% isolates were obtained from males and 30.3% from females whereas in a study by Kalidas et al and Aamal et al NFGNB isolates obtained from males was 55% and 52% respectively

whereas from females was 45% and 42% respectively.<sup>14,20,21</sup>

In our study, maximum NFGNB (37.4%) were isolated from age group of 41-60 years and minimum (1.7%) were isolated from patients above 80 years. Ridhima et al reported that the age group which was maximum infected with NFGNB was 45-60 yrs which is similar to this study.<sup>20</sup> According to Aamal et al age group in which NFGNB were more frequently isolated was 15-62 yrs.<sup>21</sup> In a study by Kalidas et al 72% isolates of NFGNB were from the age above 45 yrs and Benachinmardi et al, reported maximum NFGNB isolates were obtained from 21-50 yrs age group whereas in a study by Jayapriya et al, most of the NFGNB isolates were obtained from 21-40 yrs age group.<sup>6,14,19</sup>

In this study, 56.9% NFGNB isolates were obtained from ICU patients and 43.1% from IPD patients admitted in surgery, medicine, gynaecology and paediatric wards. The results are in concordance with studies by Juyal et al, Jayapriya et al, and Patel et al, who had reported isolation rate of NFGNB isolates from ICU samples to be 67%, 58% and 52% respectively and isolation rate from IPD as 33%, 48% and 42% respectively.<sup>11,19,22</sup>

In the present study, maximum NFGNB isolates (24.9%) were obtained from pus samples but variable isolation rates of NFGNB from pus samples have reported by other

studies: Malini et al- 62.2%, Patel et al- 58.6%, Gokale and Metgud 58.4%.<sup>15,22,23</sup> Our results are much similar to the results of Benanchinmardi et al, and Kalidas et al, who have reported it to be 22% and 27.8% respectively respectively.<sup>6,14</sup>

In this study, 23.2% NFGNB were obtained from urine samples. A study by Rajendra et al shows similar results with the isolation rate of NFGNB from urine to be 25.4%.<sup>24</sup> Jayapriya et al reported the NFGNB isolates obtained from urine to be 30.8%.<sup>19</sup> Many authors have reported very less isolation rates of NFGNB from urine samples. Benanchinmardi et al, Malini et al and Patel et al have reported NFGNB isolates obtained from urine as 11%, 11.9% and 11.8% respectively.<sup>6,15,22</sup> A study by Gokale and Metgud showed that only 8.2% NFGNB isolates were obtained from urine samples.<sup>23</sup> In the present study, 17.8% NFGNB were isolated from endotracheal secretions, 16.9% from tracheal secretions and 4.9% from sputum samples. In studies by Kalidas et al, Malini et al, Gokale and Metgud the NFGNB reported from endotracheal secretions were 18.4%, 16.4%, 6.8% and 7.8% respectively.<sup>14,15,23</sup> In a study by Malini et al, and Patel et al, NFGNB obtained from sputum samples were 6.7% and 7% respectively which also correlates with results of this study.<sup>15,22</sup> In this study, NFGNB isolated from blood samples were 4.9%. Benanchinmardi et al, and Aamal et al, have reported NFGNB isolates obtained from blood as 6% and 8% respectively thus showing similarity with the results of this study.<sup>6,21</sup> On the other hand, Sidhu et al, and Rajendra et al, have reported higher isolation rate of NFGNB from blood samples i.e.-36.3% and 24.5% respectively.<sup>18,24</sup> Very few NFGNB isolates (1.5%) were obtained from body fluids. Similar results have been reported by other studies in which NFGNB isolated from body fluids were 1.5%, 2.4% and 2.3% respectively.<sup>11,15,23</sup>

In the present study, *P. aeruginosa* was the most frequently isolated NFGNB as 52.6% of total NFGNB isolates were of *P. aeruginosa*. Various authors have reported similar prevalence of *P. aeruginosa* in their studies: 53.8%, 53%, 50.2%, 58.9% and 56.9%.<sup>13-15,25</sup> Higher prevalence of *P. aeruginosa* has also been reported by some authors: 72.6% 78.9%, 82.3% and 76.9%.<sup>4,5,22,23</sup> Lesser prevalence of *P. aeruginosa* has been reported by Samanta et al and Juyal et al i.e.- 26% and 38.2% respectively.<sup>11,25</sup> *A. baumannii* was the second most frequently isolated NFGNB as 31.7% of total NFGNB isolates were of *A. baumannii*. Different authors have reported similar prevalence of *A. baumannii* in their studies: 39%, 30.3%, 30.5%.<sup>2,6,19</sup>

In the present study, *S. maltophilia* showed the prevalence of 4.1% which is very close to the results reported by Jayapriya et al who had reported it to be 4.5%.<sup>19</sup> Kalidas et al and Rajendra et al have reported the prevalence of *S. maltophilia* as 3% and 3.6% respectively.<sup>14,24</sup> In this study, *A. lwoffii* showed the prevalence of 4.1% which is very close to the results

reported by Kalidas et al who reported it to be 5.4%.<sup>14</sup> However, Juyal et al and Rajendra et al have reported the prevalence of *A. lwoffii* as 13.8% and 9.1% respectively which is slightly higher as compared to this study.<sup>6,24</sup> *B. cepacia* showed the prevalence of 2.5% in this study which is very near to the results reported by Jayapriya et al who reported it to be 3.2% whereas Kalidas et al, had reported the prevalence of *B. cepacia* to be 6.9% which is slightly higher as compared to this study.<sup>6,19</sup> *P. fluorescens*, *P. putida*, *P. stutzeri*, *A. faecalis*, *A. xylooxidans*, *S. paucimobilis*, *B. diminuta* were less frequently isolated NFGNB accounting to the total prevalence of 4.7%. Various studies around the globe also reported very less prevalence of these NFGNB isolates.<sup>4,15,26-29</sup>

*P. aeruginosa* was found resistant to most of the commonly used antimicrobial agents. It was found highly resistant to ceftazidime (60.9%) and cefepime (57.8%) which is similar to other studies. Juyal et al, Kalidas et al and Patel et al, have reported resistance of *P. aeruginosa* for ceftazidime to be 68.8%, 71.3% and 75.4% respectively.<sup>6,14,22</sup> Juyal et al reported resistance towards cefepime as 61.4% whereas very low level of resistance was recorded by Sadhna et al, 28% and Bimla and Rekha -25.3%.<sup>6,30,31</sup> Higher resistance to cephalosporins might be due to production of ESBL's by this bacteria. In this study, piperacillin-tazobactam was effective antibiotic as only 35.4% resistance was recorded for this antibiotic. This is almost similar to studies by Kalidas et al and Patel et al who have recorded it as 46.5% and 24.1% respectively.<sup>14,19</sup> Sensitivity of *P. aeruginosa* to imipenem in this study was 71.3%. Various other studies have also reported imipenem as effective antibiotic to treat infections caused by *P. aeruginosa* with sensitivity of 94%, 91% and 89% respectively.<sup>6,14,22</sup> In our study, resistance towards meropenem was higher as compared to imipenem. Overexpression of the MexAB-Opr M efflux system is known to affect meropenem efficacy but not that of imipenem. In addition, the MexCD- OprJ and Mex XY-Opr M efflux systems may be involved in reduced susceptibility to meropenem.<sup>32</sup> Polymyxin B and colistin were the most effective antimicrobial agents against *P. aeruginosa* as 100% sensitivity was recorded for them in this study and various other studies.<sup>14,19,33,34</sup>

*A. baumannii* isolates were found to be extremely resistant to ceftazidime (96.6%); cefepime (94.8%) and piperacillin-tazobactam (94.8%). Higher level of resistance was also recorded for cotrimoxazole (92.2%), ampicillin-sulbactam (74.2%) amikacin (89.7%) and ciprofloxacin (88.7%). Resistance towards imipenem and meropenem was recorded as 60.4% and 68.1% respectively. This correlates with the studies by Kalidas et al and Jaggi et al and who have reported resistance towards imipenem and meropenem to be 65.4% and 62.6% respectively.<sup>14,35</sup> Lower resistance was seen in polymyxin B and colistin in this study. Kalidas et al, Taneja et al and Nahar et al and also recorded 10.5%, 3.5% and 5% resistance of *A. baumannii* towards



colistin.<sup>14,26</sup> *A. lwoffii* was found much more susceptible to antibiotics as compared to *A. baumannii*. Many authors have reported *A. lwoffii* as a drug susceptible organism as compared to *A. baumannii*.<sup>36-38</sup> All isolates of *S. maltophilia* were resistant towards ceftazidime, cefepime, imipenem and meropenem. However, it was found that only 26.6% isolates were resistant to piperacillin-tazobactam and 13.3% towards gentamicin and amikacin. All isolates were sensitive to cotrimoxazole. These results are in correlation with the results reported by Kalidas et al Nonika et al and Paez et al.<sup>14,39,40</sup> In the present study, *B. cepacia* showed very good sensitivity towards cefepime (77.7%), imipenem (66.6%), meropenem (77.7%). All isolates were sensitive to piperacillin-tazobactam and cotrimoxazole. All isolates were resistant to polymyxin B and colistin as *B. cepacia* shows intrinsic resistant towards these drugs.<sup>1,2</sup> Kalidas et al and Sidhu et al have also reported similar antibiogram of *B. cepacia* in their studies.<sup>14,18</sup>

## CONCLUSION

It may be concluded that growth of NFGNB cannot be overlooked and should be confronted with high index of suspicion. Precise identification of these bacteria upto genus and species level, imperative clinic-microbiological correlation and careful antibiotic prescription shall go a long way in improving clinical outcomes of patients. This study also gives an alarming sign towards high prevalence of multi drug resistant NFGNB. It is noteworthy that as these bacteria also have a great potential to survive in hospital environment therefore, improved antibiotic stewardship, good housekeeping, equipment decontamination, strict protocols for hand washing, isolation procedures need to be implemented to prevent emergence and spread of multidrug resistant NFGNB in health care settings.

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