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Original Research Article.

Prevalence of Non Fermenting Gram Negative Bacilli with Their in vitro Antibiotic Sensitivity Profile in RIMS, Ranchi

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ABSTRACT

Background: Non fermenting Gram Negative Bacilli are diverse and complex group of bacteria that possess very few defined characteristics. They are aerobic, non-fermenting Gram negative bacilli which were initially considered as contaminants but have come up with life threatening infections in hospitals as multidrug resistant organisms posing a threat because of their inherent and acquired drug resistance nature.

Aims: Isolation and identification of NFGNB in clinical samples and determination of their antibiotic sensitivity profile.

Materials and Methods: The study was conducted in the Department of Microbiology, RIMS, Ranchi from February 2017-July 2017. Various clinical samples reaching the Bacteriology section of the Department of Microbiology were processed and NFGNB were isolated and identified using standard procedure and their antibiotic susceptibility was performed.

Results: A total of 3581 samples were received out of which 2246 were culture positive and 217 were identified as NFGNB. The isolation rate of NFGNB was 9.6%. Number of males affected by NFGNB was 121 and that of females was 96. Analysed by specimen NFGNB were isolated from 91 urine, 74 pus, 11 ear swab, 6 sputum, 8 body fluid, 21 blood culture and 6 catheter tip samples. Urine was most common specimen accounting for 42% followed by pus (34%), blood (9%), ear swab (5%), body fluid (4%), sputum and catheter tip (3%each). The clinical samples from indoor patients yielded highest percentage of NFGNB (38%) followed by ICU patients (36%) and outdoor patients (26%). Among the NFGNB isolated *Pseudomonaas aeruginosa* (51%) was the most common followed by *Acinetobacter baumanii* (22%), *Pseudomonas* spp (19%), *Acinetobacter* spp, *Stenotrophomonas maltophila*, *Burkholderia cepacia* (2% each), *Ralstonia* spp & *Sphingobacterium* spp (1%). Non fermenters were highly sensitive to Imipenem accounting for 91.5% followed by Piperacillin-tazobactam (71.5%), cefoperazone sulbactam (67.7%) & Amikacin (55.6%) on an average.

Conclusion: NFGNB considered being contaminants in the past have now emerged as important health care associated infections. In our setting Imipenem can be used for the preliminary treatment of infections caused by nonfermenters. As these organisms are important opportunistic and nosocomial pathogens causing infections in immunocompromised patients, better infection control policies in our settings and its implementation is a must.

Keywords: Nonfermenting Gram Negative Bacilli, *P. aeruginosa, Acinetobacter baumanii*, *Pseudomonas* spp, *Acinetobacter* spp., Imipenem.

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INTRODUCTION

The group of organisms referred to as Nonfermenting gram negative bacilli (NFGNB) are taxonomically diverse group of aerobic, non sporing bacilli that either do not utilize glucose as a source of energy or utilize it oxidatively.[1] They occur in the environment as saprophytes, previously considered as contaminants or commensals.[2,3]

Recent literature suggests that these organisms are associated with certain life threatening infections such as septicaemia, pneumonia, urinary tract infections, meningitis, surgical site infections, ventilator associated pneumonia, wound infection, osteomyelitis etc.[3] NFGNB account for around 15% of all bacterial isolates in a clinical microbiology lab of a hospital setting.[4] The emergence of NFGNB as important health care associated pathogens can be attributed to indiscriminate and injudicious use of broad spectrum antibiotics as these organisms show resistance to drugs routinely administered in clinical practice in health care settings.[5] NFGNB have innate resistance to certain antibiotics and are also known to produce extended spectrum beta lactamases

(ESBL) and metallo beta lactamases (MBL).[6] There is paucity of literature from our area on NFGNB, their presence in various clinical samples and their antibiotic sensitivity. Therefore, this study was undertaken to know the prevalence of these organisms in all the clinical samples along with their clinical significance. As these groups of organisms are intrinsically resistant to many antibiotics and have also developed acquired resistance to many drugs it is imperative to know their antibiotic sensitivity profile so as to fulfill the goals of antibiotic stewardship and help in reducing the cost of treatment on both patients as well as our health care settings.

MATERIALS AND METHODS

The study was a cross-sectional study done in the bacteriology section of Department of Microbiology, RIMS, Ranchi for a period of 6 months from February 2017- July 2017. All the clinical samples received at the bacteriology section were inoculated on blood agar, Mac Conkey Agar, CLED Agar and incubated at 37˚ C for 48hrs. All the isolates that were nonlactose fermenting and showed alkaline/ no change (K/NC) reaction on Triple Sugar Iron (TSI) Agar slant were provisionally considered as NFGNB and were further identified using the standard protocol for identification. The tests performed were Gram stain for morphology, hanging drop for motility, oxidase test, catalase test, citrate utilization, oxidative-fermentative test (Hugh-Leifson medium) for glucose, lactose, sucrose, maltose, mannitol and xylose, gelatin liquefaction, lysine ornithine decarboxylation, arginine dihydrolase test, growth at 42˚C and 44˚C.

Antimicrobial sensitivity was performed using Kirby-Bauer disc diffusion method on Mueller-Hinton Agar using commercially available antimicrobial discs (Hi-media). The different antibiotics used were Ampicillin (10µg), Ciprofloxacin (5µg), cotrimoxazole (1.25/23.75µg), Cefotaxime (30µg), cefpodoxime (10µg), Gentamicin (10µg), Levofloxacin (5µg), Nalidixic Acid (30µg), Nitrofurantoin (300µg), Amoxicillin (25µg), Netilmycin (30µg), Amikacin (30µg), Ampicillin-sulbactam (10/30µg), Imipenem (10), Piperacillin- tazobactam (100/10µg). The results were interpreted using Clinical and Laboratory Standard Institute guidelines.[7] Controls used were *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853.

Fig 1: Sex distribution of NFGNB infection in clinical samples

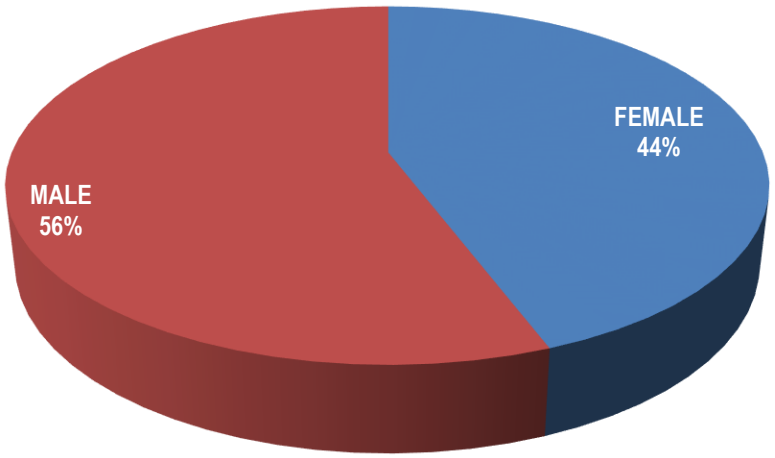
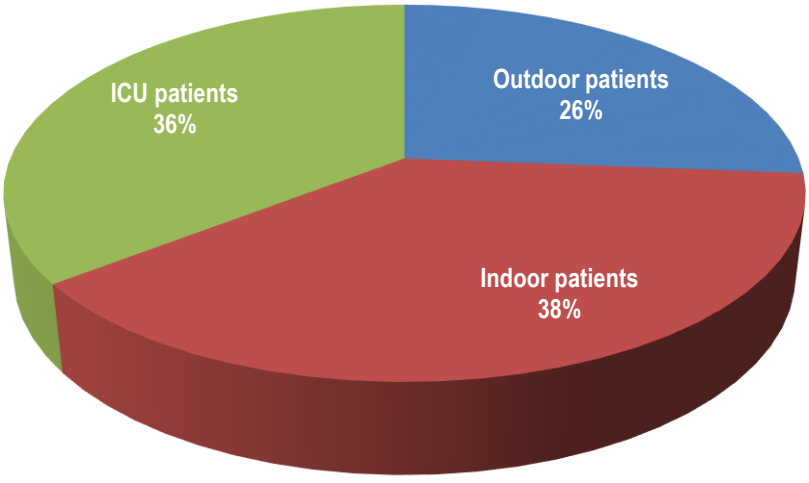


Fig 2: Distribution of NFGNB in patients



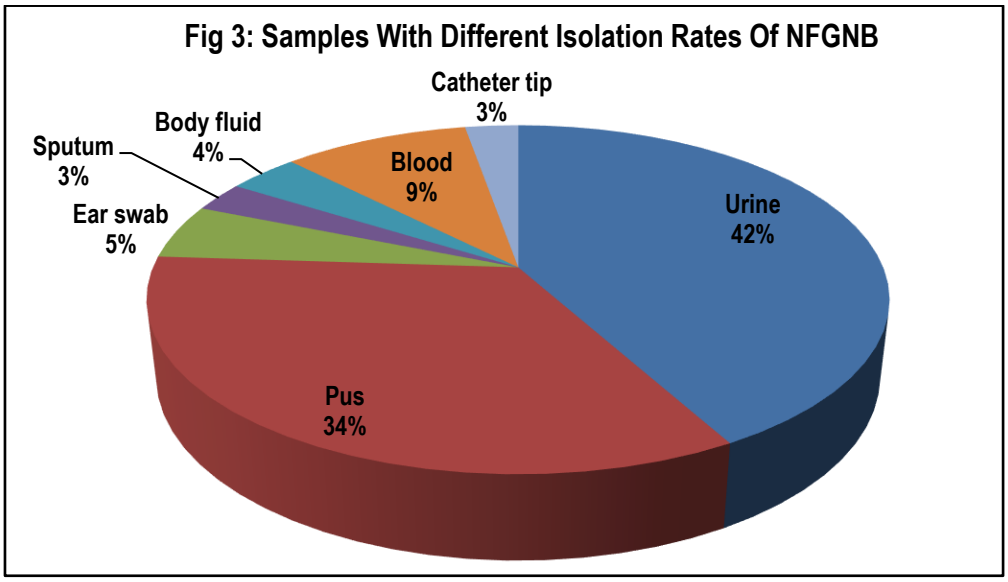


Fig 3: Samples With Different Isolation Rates Of NFGNB
Catheter tip 3%
Body fluid 4%
Sputum 3%
Blood 9%
Ear swab 5%
Urine 42%
Pus 34%

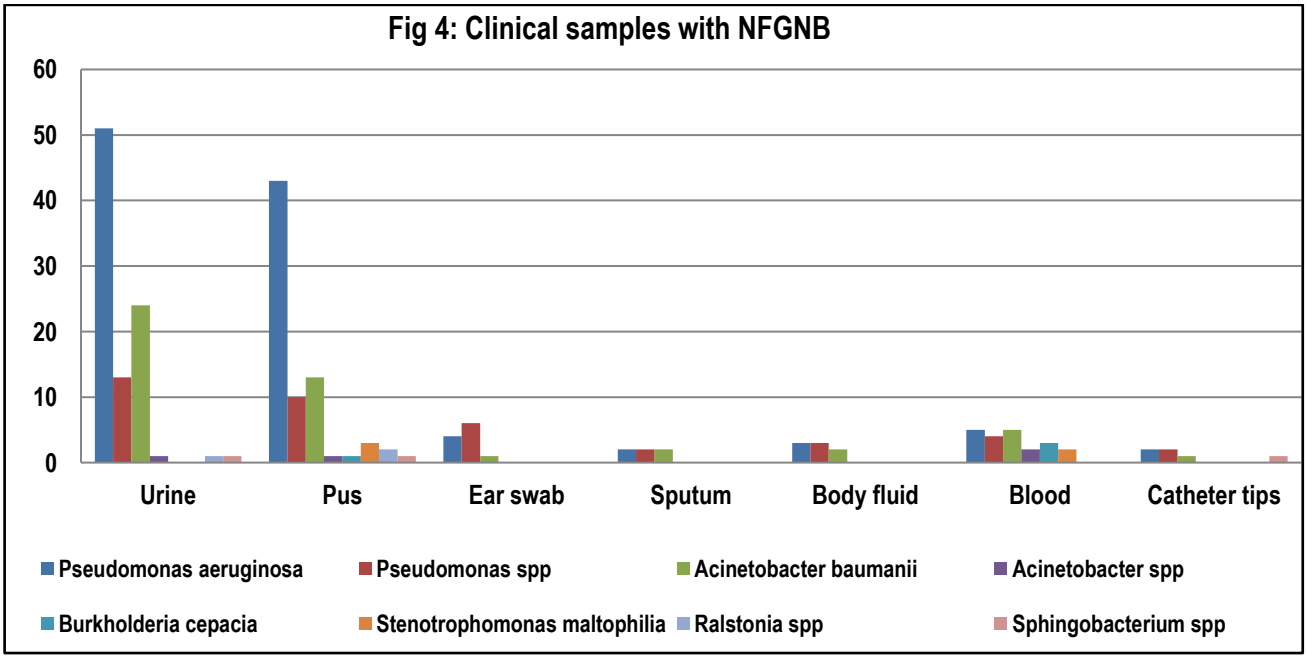


Fig 4: Clinical samples with NFGNB
60
50
40
30
20
10
0
Urine
Pus
Ear swab
Sputum
Body fluid
Blood
Catheter tips
Pseudomonas aeruginosa
Pseudomonas spp
Acinetobacter baumanii
Acinetobacter spp
Burkholderia cepacia
Stenotrophomonas maltophilia
Ralstonia spp
Sphingobacterium spp

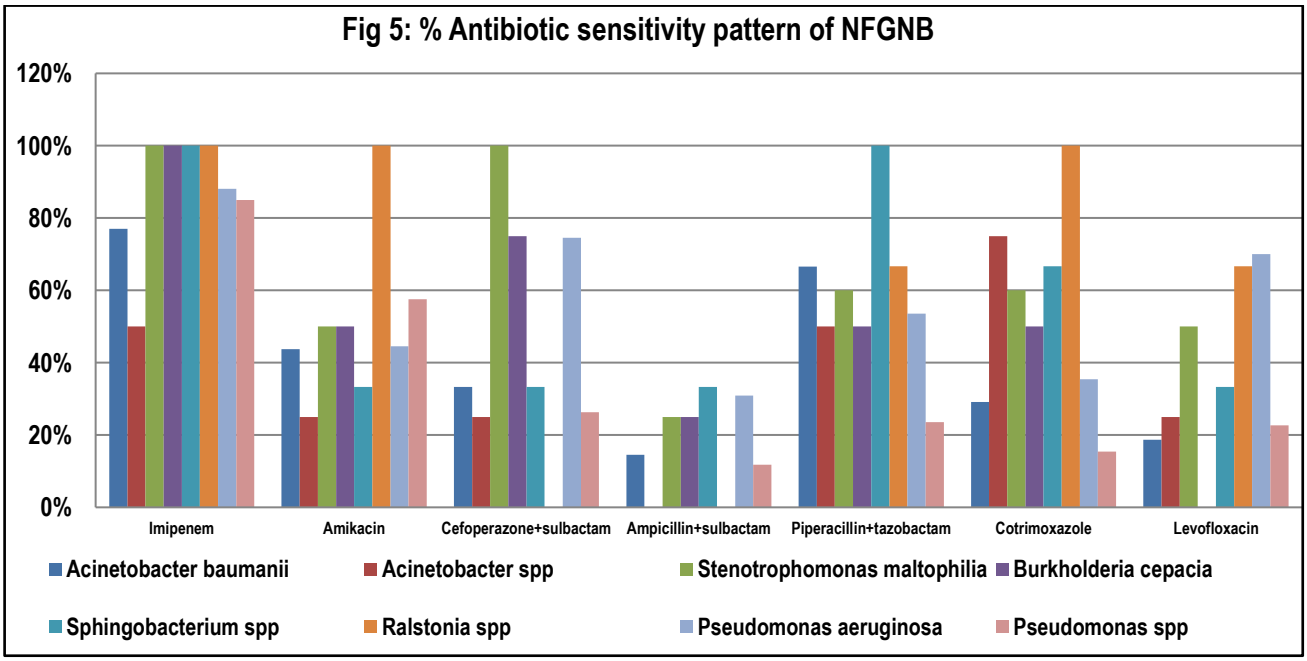


Fig 5: % Antibiotic sensitivity pattern of NFGNB
120%
100%
80%
60%
40%
20%
0%
Imipenem
Amikacin
Cefoperazone+sulbactam
Ampicillin+sulbactam
Piperacillin+tazobactam
Cotrimoxazole
Levofloxacin
Acinetobacter baumanii
Acinetobacter spp
Stenotrophomonas maltophilia
Burkholderia cepacia
Sphingobacterium spp
Ralstonia spp
Pseudomonas aeruginosa
Pseudomonas spp

Table 1: Non fermenters isolated from different clinical samples

Samples	PA	PS	AB	AS	BC	SM	RS	SS	Total
Urine	51	13	24	1	-	-	1	1	91
Pus	43	10	13	1	1	3	2	1	74
Ear swab	4	6	1	-	-	-	-	-	11
Sputum	2	2	2	-	-	-	-	-	6
Body fluid	3	3	2	-	-	-	-	-	8
Blood	5	4	5	2	3	2	-	-	21
Catheter tips	2	2	1	-	-	-	-	1	6
Total	110	40	48	4	4	5	3	3	217

PA: P. aeruginosa; PS: Pseudomonas spp.; AB; Acinetobacter baumanii; AS: Acineto. Spp; BC: Burkholderia cepacia; SM: Stenotrophomonas maltophila; RS: Ralstonia spp; SS: Sphingobacterium spp.

Table 2: Antibiotic sensitivity of non fermenting Gram negative Bacilli

Antibiotics (µg)	AB		AS		SM		BC		SS		RS	
	S%	R%	S%	R%	S%	R%	S%	R%	S%	R%	S%	R%
Imipenem (10)	77	23	50	50	100	0	100	0	100	0	100	0
Amikacin (30)	43.7	36.2	25	75	50	50	50	50	33.3	66.7	0	100
Cefo+ sul (75/30)	33.3	66.7	25	75	100	0	75	25	33.3	66.7	0	100
Ampi+sul (10/10)	14.5	85.5	0	100	25	75	25	75	33.3	66.7	0	100
Pipera+tazo (100/10)	66.6	33.4	50	50	60	40	50	50	100	0	66.7	33.3
Cotrim (1.25/23.75)	29.1	70.8	75	25	60	40	50	50	66.7	33.3	100	0
Levo (5)	18.7	81.2	25	75	50	50	0	100	33.3	66.7	66.7	33.3

AB; Acinetobacter baumanii; AS: Acineto. Spp; BC: Burkholderia cepacia; SM: Stenotrophomonas maltophila; RS: Ralstonia spp; SS: Sphingobacterium spp.

Table 3: Antibiotic sensitivity of *Pseudomonas aeruginosa and Pseudomonas spp.*

Antibiotics (µg)	*Pseudomonas aeruginosa*		*Pseudomonas spp*	
	S%	R%	S%	R%
Imipenem (10)	88.1	11.9	85	15
Amikacin (10)	44.5	55.5	57.5	42.5
Cefopera+ sulbac (75/30)	74.5	25.5	26.3	73.7
Ampi+ sulbactam (10/30)	30.9	69.1	11.8	88.2
Pipe+ tazo (100/10)	53.6	46.4	23.6	76.4
Cotrimoxazole (1.25+23.75)	35.4	64.6	15.4	84.6
Levofloxacin (5)	70	30	22.7	77.3

RESULTS

A total of 3581 samples were received out of which 2246 were culture positive and 217 were identified as NFGNB. The isolation rate of NFGNB was 9.6%.Number of males was 121 and that of females was 96 the ratio being 1.26 as depicted in Fig 1. The clinical samples from indoor patients yielded highest percentage of NFGNB (38%) followed by ICU patients (36%) and outdoor patients (26%). (Fig 2)

Analysed by specimen NFGNB were isolated from 91 urine, 74 pus, 11 ear swab, 6 sputum, 8 body fluids, 21 blood culture and 6 catheter tip samples. Urine was most common specimen accounting for 42% followed by pus (34%), blood (9%), ear swab (5%), body fluid (4%), sputum and catheter tip (3%each). (Table 1, Fig 3) Among the NFGNB isolated *Pseudomonas aeruginosa (*51%) was the most common followed by *Acinetobacter baumanii (*22%), *Pseudomonas spp (*19%), *Acinetobacter* spp*, Stenotrophomonas maltophila, Burkholderia cepacia (2% each), Ralstonia* spp. and *Sphingobacterium* spp.(1%) each. (Table 1, Fig 4)

The antibiotic sensitivity pattern of Non fermenters showed them to be highly sensitive to Imipenem accounting for 91.5% on an average followed by Piperacillin-tazobactam (71.5%), cefoperazone-sulbactam (67.7%) & Amikacin (55.6%) on an average. Most of the organisms showed resistance to Cefotaxime and Cotrimoxazole. (Table 2, 3, Fig 5)

DISCUSSION

Previously considered as commensals or contaminants, these groups of organisms have shown themselves to be of pathogenic potential as they have been frequently isolated from clinical samples from hospitalised and immunocompromised patients. They have gained importance as causative agents of nosocomial infections having intrinsic as well as acquired resistance to different classes of drugs used frequently in clinical practice by the medical practitioners.

Out of 3581 samples received in our bacteriology section 217 were identified as NFGNB so that the isolation rate in our set up was 9.6% which is different from studies done by Bruno et al.[8], Malini et al.[9], Benachinmardi et al.[10] where the isolation rate was 2.18%, 3.58% and 4.5% respectively. Our study had isolation rates parallel to studies done by Samanta et al.[11] where the isolation rate was 10%. Eltahawy and Khalaf[12], Vijaya et al.[2] and Sidhu et al.[13] have reported higher isolation rates of 16%, 21.8% and 45.9% respectively.

Urine was the most common sample from which NFGNB were isolated. 91 urine samples (42%) yielded NFGNB followed by pus samples which accounted for 74 (34%). The common organisms isolated from urine samples were P*seudomonas aeruginosa* (51), *Acinetobacter baumanii* (24), *Pseudomonas* spp. (13). Those isolated from pus were *Pseudomonas aeruginosa* (43) *Acinetobacter baumanii* (13) *Pseudomonas* spp (10). *Acinetobacter baumanii* (5) and *Pseudomonas aeruginosa* (5) were frequently isolated from blood cultures.

Maximum number of isolates from our study were isolated from urine samples (42%) which is different from that observed in other studies where most of the isolates were from pus samples.[5,6,14,15] NFGNB isolated from indoor patients was more than the outdoor patients which is in concordance to other studies done by Benachinmardi *et al.*[10], Jayanti and Jeya[16] and Juyal.[3] *Pseudomonas aeruginosa* and *Acinetobacter baumanii* were the most common NFGNB isolated from clinical samples which is similar to studies done by others.[9]

Nosocomial pathogens show resistance pattern which varies world-wide, from country to country and within the same country at different time periods.[17]

In our study most of the NFGNB were sensitive to Imipenem followed by Piperacillin–tazobactam, cefoperazone- sulbactam, and Amikacin. *Pseudomonas aeruginosa* was 88.1% and *Pseudomonas spp* was 85%sensitive to Imipenem. *Burkholderia, Sphingotrophomonas, Ralstonia were* 100% sensitive to Imipenem. *Acinetobacter baumanii* was 77.5% sensitive to Imipenem. Imipenem was followed by piperacillin-tazobactam sensitivity wherein *Sphingobacterium* was 100% sensitive, *Pseudomonas aeruginosa* was sensitive 53.6% and *Acinetobacter baumanii* showed 66.6% sensitivity to PTZ.

Pseudomonas spp showed good sensitivity to cefoperazone-sulbactam (74.5%). Levofloxacin showed good sensitivity to *Pseudomonas aeruginosa* (70%). The sensitivity shown by Cotrimoxazole showed to *Stenotrophomonas* (60%)*and Sphingobacterium (*66.6%*)*.

Other studies showed good sensitivity to amikacin[2,5,9] whereas some other studies showed 60-70% resistance to amikacin and ciprofloxacin.[3,17,18] Imipenem showed very good sensitivity as in other studies by Malini *et al* [9], Gupta[19], Gladstone *et al*.[20],Hodiwala.[21]

CONCLUSION

Pseudomonas aeruginosa and Acinetobacter baumanii were the most common NFGNB isolated from various clinical samples in our study. They were associated with a number of infections like urinary tract infection, wound infection, surgical site infection, ventilator associated pneumonia, septicaemia and respiratory tract infections. *Pseudomonas aeruginosa* shows good *sen*sitivity to Imipenem, Piperacillin- tazobactam and cefoperazone- sulbactam. *Acinetobacter* also showed good sensitivity to Imipenem but not to other drugs due to resistance to other drugs. Most of the NFGNB have shown good response to Imipenem thereby making it the drug of choice in our healthcare setting. But they were resistant to other broad spectrum antibiotics like cefotaxime and cefpodoxime. Also amikacin that has proved to be a good choice against these NFGNB is mostly resistant in our setting. Therefore it becomes necessary to keep an eye on the sensitivity pattern to guide the clinician for better patient care and management. As they are nosocomial pathogens they survive in hospital environment and are responsible for propagation of infection, therefore formulating infection control policies and following antibiotic stewardship along with its implementation is a must in our healthcare setting to prevent the emergence of drug resistant strains and their spread. Its high time to conserve our resources and our antibiotics to combat the growing power of drug resistant organisms before they outreach complete resistance.

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