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Microbiology

KEY WORDS:

IDENTIFICATION OF NON -FERMENTER GRAM NEGATIVE BACILLI (NFGNB) AND ITS ANTIBIOTIC SUSCEPTIBILITY PATTERN IN VARIOUS CLINICAL ISOLATES AT A TERTIARY CARE HOSPITAL.

Dr Anu sharma* Assistant Professor, Microbiology department Dr V.M. Government Medical college, solapur, Maharashtra. * Corresponding Author

Dr K.V. Ingole Professor,Microbiology department Dr V.M. Government Medical college, solapur, Maharashtra.

Dr Abhilasha belpatre Post graduate student., Microbiology department Dr V.M. Government Medical college, solapur, Maharashtra.

ABSTRACT

Background: Non fermenter gram negative (NFGNB) have emerged as important pathogen responsible for hospital associated infections, as these bacilli show multi drug resistance , therefore it is important to correctly identify and know there antibiotic susceptibility pattern for better patient care.
Aim & objective of study: To isolate and identify non fermenter gram negative bacilli from various clinical isolates and to know the distribution pattern in various samples along with antibiotic susceptibility pattern of the isolates.
Material & method: NFGNB isolated from various clinical samples like pus, urine, blood and respiratory specimen which were received for culture and sensitivity in Microbiology department at a tertiary care hospital were included in the study.
Results: Among 1566 clinical sample received, NFGNB isolates were 200(12.77%). . Among 200(12.77%) NFGNB isolates 95 (47.5%) isolates were from pus followed by 55 (27.5%) isolates from sputum, 23(11.5%) isolates from urine, 17 (8.5%) isolates from blood, 7(3.5%) isolates from endotracheal secretions and 3 (1.5%) isolates from pleural fluid. Out of 200 NFGNB isolates, *Pseudomonas aeruginosa* were the commonest organism constituting 112(56%) of the total isolates, followed by *Acinetobacter baumannii* which constituted for 71 (35.5%) , Pseudomonas fluorescens- 7 isolates (3.5%), Acinetobacter lwoffii- 6 isolates (3%), *Burkholderia cepacia* complex -2 isolates (1%) and *Sphingomonas paucimobilis* – 2 isolates (1%). Overall all NFGNB (n=200) showed high sensitivity to colistin 100%, imipenem 70.5%, amikacin 65.5% and piperacillin-tazobactam 64% however maximum resistance was noted for ciprofloxacin 61.5%. .

INTRODUCTION

Non fermenter gram negative bacilli (NFGNB) are group of aerobic, non-sporing bacilli that either do not use carbohydrate as a source of energy or degrade them through metabolic pathways other than fermentation[1]..These are ubiquitous in nature particularly in soil and water. NFGNB are known to account for about 15% of all bacterial isolates from clinical Microbiology laboratory[2].

This group includes organisms from diverse genera like *Pseudomonas, Acinetobacter, Alcaligens, Stenotrophomonas, Burkholderia cepacia* complex etc. Currently *Pseudomonas aeruginosa* and *Acinetobacter baumanii* are most commonly isolated non fermenters pathogenic for humans whereas infections caused by other species are relatively infrequent[3].

In the hospital environment they may be isolated from instruments such as ventilator machines, humidifiers, mattresses and other equipment's. All these organisms have the potential to spread horizontally on fomites or hands of the health care workers[3,4,5]. The emerging antimicrobial resistance and epidemiological complexity have made NFGNB the remarkable organisms[8]. Risk factors for NFGNB infections includes immunosuppression, neutropenia, mechanical ventilation, cystic fibrosis, indwelling catheters, invasive diagnostic and therapeutic procedures, prolonged hospital stay, broad spectrum antibiotics use .

The emergence of multidrug resistance for NFGNB is of great concern to clinicians, due to high innate resistance shown by NFGNB to commonly used antibiotics, proper identification and determination of antibiotic susceptibility pattern is very important for proper management of infection caused by these pathogens[3]. Therefore early identification and institution of appropriate treatment is necessary to reduce morbidity and mortality due to these organisms in hospitalized patients[6].

The present study was therefore conducted to identify NFGNB from various clinical specimens and to determine their antibiotic susceptibility pattern this may provide necessary information for formulation of hospital antibiotic policy and also help to prevent the spread of multi drug resistant strains in the community.

MATERIAL AND METHODS: NFGNB isolated from various clinical samples like pus, blood, urine and respiratory specimens received for culture and sensitivity in department of Microbiology from Medicine, Surgery and Orthopedics department at a tertiary care hospital were included in the study. Duration of study was between December 2015 to July 2017. Clinical isolates of NFGNB from samples like pus, blood, urine and respiratory specimens were included in study while samples other than above foresaid and mixed growth of more than 3 types (probably contaminant sample) were excluded from the study. All the samples were collected under aseptic precautions. Permission from ethical committee was taken. Smears were made from the specimen of pus, urine and respiratory specimen (except blood), after staining, smears were examined microscopically to look for pus cells and Gram negative bacilli and coco-bacilli. The specimens were inoculated on the Blood agar (BA), Mac Conkey agar (MA). In case of urine sample was inoculated on to cysteine lactose electrolyte deficient (CLED) medium for semi-quantitative culture. The medium were incubated at 37ºC overnight thereafter plates were examined for the growth. Plates with growth of NFGNB on MA were inoculated on TSI medium. Those organisms which failed to acidify the butt of TSI medium, were presumptively identified as NFGNB and were subjected to preliminary tests and further identification were done using standard biochemical tests with standard positive and negative controls[7,8].The antibiotic susceptibility testing of the isolates was done by Kirby- Bauer disk diffusion method on Mueller Hinton agar. Isolates were grown in suitable liquid medium (peptone water) by incubating at 37ºC for 4 hours and turbidity was matched with 0.5 Mac Farland standards. Then lawn culture was done on Mueller Hinton agar plate and commercial antibiotic disks were placed. The plates were incubated at 37ºC overnight and on the next day the zones of inhibition were measured and susceptibility/ resistance interpreted according to CLSI guidelines 2015[19].All the discs were procured commercially (Hi-media laboratories limited).The diameter of the zone of inhibition was measured and interpreted according to the CLSI 2015 guidelines[19].

PHOTO-1 Pigmented Colonies Of *Pseudomonas Aeruginosa* On Nutrient Agar.

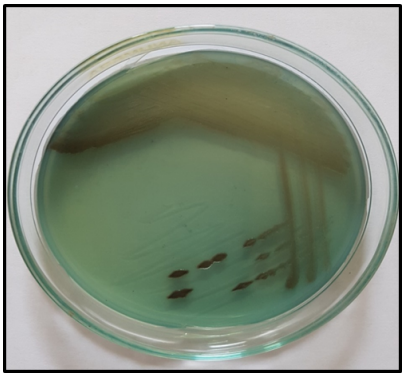
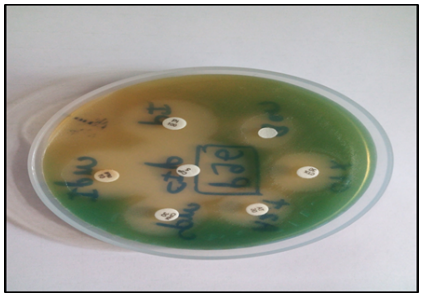


PHOTO-2 *Acinetobacter baumanii* growth on blood agar



PHOTO -3 Antibiotic Sensitivity Pattern Of *Pseudomonas Aeruginosa*

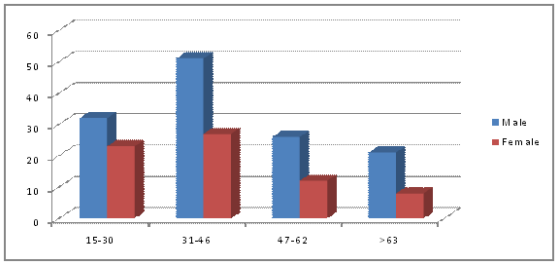


RESULTS AND OBSERVATION: A total of 1566 various clinical samples were received in Microbiology department at a tertiary care hospital. Among 1566 clinical sample received, NFGNB isolates were 200(12.77%). Majority of NFGNB isolates were from males which constituted 130 (65%) while 70 (35%) were from females. Maximum isolates were from patient belonging to age group 31-46 years followed by 15-30 years in both gender as shown in table-1 and graph-1.

TABLE-1 - Age And Sex Wise Distribution Of NFGNB

Age group (years)	Total number if isolates in males (n=130)	Total number of isolates in females (n=70)	Percentage (%)
15-30	32	23	27.5
31-46	51	27	39
47-62	26	12	19
>63	21	08	14.5

GRAPH -1: Age and sex wise distribution of isolated NFGNB



From the 200 NFGNB isolated from various clinical samples, maximum NFGNB 87 (43.5%) were from Surgery department of which maximum 80 were from Surgery ward followed by Surgery ICU 06 and 01 from OPD. Eighty two (41%) NFGNB were from Medicine department of which maximum 60 were from Medicine ward followed by Medicine ICU 17 and 05 from Medicine OPD. Thirty one (15.5%) NFGNB were from Orthopedics department of which maximum 28 were from Orthopedics ward followed by 03 from OPD as shown in table-2.

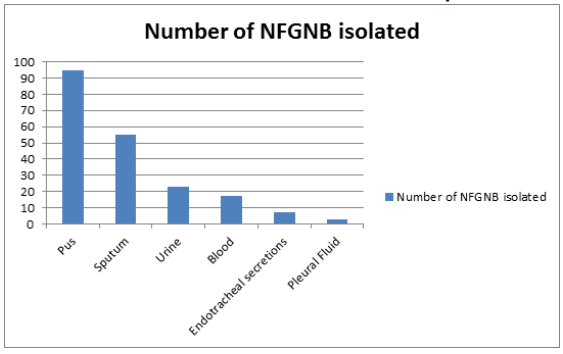
TABLE-2: Distribution of NFGNB isolates from OPD and IPD

Department	IPD		OPD	Total	Percentage (%)
	Ward	ICU			
Medicine	60	17	05	80	41
Surgery	80	06	01	87	43.5
Orthopedics	28	-	03	31	15.5

Distribution of NFGNB according to clinical samples

Among 200(12.77%) NFGNB isolates 95 (47.5%) isolates were from pus followed by 55 (27.5%) isolates from sputum, 23(11.5%) isolates from urine, 17 (8.5%) isolates from blood, 7(3.5%) isolates from endotracheal secretions and 3 (1.5%) isolates from pleural fluid as shown in graph-2.

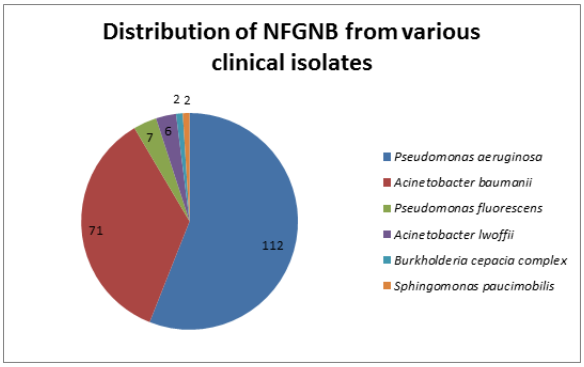
GRAPH-2: Distribution of NFGNB from various specimens



Different isolates of NFGNB

Out of 200 NFGNB isolates, *Pseudomonas aeruginosa* were the commonest organism constituting 112(56%) of the total isolates, followed by *Acinetobacter baumannii* which constituted for 71 (35.5%), *Pseudomonas fluorescens*- 7 isolates (3.5%), *Acinetobacter lwoffii*- 6 isolates (3%), *Burkholderia cepacia* complex -2 isolates (1%) and *Sphingomonas paucimobilis* – 2 isolates (1%) as shown in graph-3.

GRAPH-3: Showing NFGNB Distribution in Various Clinical isolates



Among 112 (56%) of *Pseudomonas aeruginosa*, 65(58.03%) were from pus sample followed by 25 (22.32%) from sputum, from urine 13(11.60%), endotracheal secretion 4(3.57%), *pleural fluid* 3 (2.67%), blood 2 (1.7%), whereas out of 7 isolates of Pseudomonas fluorescens 3 (42.85%) and 4 (57.14%) were from pus and sputum respectively.

Out of 71 (35.5%) of *Acinetobacter baumanii* 24 (33.80%) were from pus followed by 20 (28.16%) from sputum, 14 (19.71%) from blood, 10 (14.08%) from urine and 3(4.22%) from endotracheal secretions.

Out of 6 (3%) *Acinetobacter lwoffii* 3 (50%) were from pus sample followed by 2 (33.33%) were from sputum and 1 (16.66%) from blood.

2(1%) isolates of *Burkholderia cepacia* complex were from sputum sample. *Sphingomonas paucimobilis* 2 (1%) isolates were from sputum sample as shown in table-3.

TABLE-3: Distribution Of Non-fermenter Isolates In Various Specimens:

Species	Sputum	Pleural fluid	Endotracheal secretion	Pus	Blood	Urine
Pseudomonas aeruginosa	25	03	04	65	02	13
Pseudomonas fluorescens	04	-	-	03	-	-
Acinetobacter baumanii	20	-	03	24	14	10
Acinetobacter lwoffii	02	-	-	03	01	-
Burkholderia cepacia complex	02	-	-	-	-	-
Sphingomonas paucimobilis	02	-	-	-	-	-
Total	**55**	**03**	**07**	**95**	**17**	**23**
Percentage (%)	**18.77**	**1.5**	**3.5**	**47.5**	**8.5**	**11.5**

Antibiotic susceptibility pattern of Pseudomonas species

Pseudomonas species (n=119) were found to be 100% sensitive to colistin. Among *Pseudomonas species* 72.32% of *Pseudomonas aeruginosa* were found to be sensitive to imipenem followed by 69.64% sensitive for amikacin and 67.85% sensitive for ceftazidime, Whereas maximum resistance was noted for ciprofloxacin, piperacillin and in aztreonam 55.36%, 44.65% and 41.37% respectively as shown in table-4.

All the strains of *Pseudomonas fluorescens* were 100% sensitive to colistin whereas the sensitivity of *Pseudomonas fluorescens* isolates for imipenem, amikacin, ceftazidime, piperacillin, piperacillin-tazobactam, cefaperazone were 85.71%,71.42%,57.14%, 57.14%,57.14% and 57.14% respectively. Maximum resistance in *Pseudomonas fluorescens* was noted for gentamicin 71.43% followed by ciprofloxacin 57.15% and aztreonam 57.15% as shown in table-4.

TABLE-4: Antibiotic sensitivity pattern of *Pseudomonas species*

Drugs (µg)	*Pseudomonas aeruginosa* (n=112)		*Pseudomonas fluorescens* (n=7)	
	S	%	S	%
Ceftazidime (30)	76	67.85	4	57.14
Gentamicin (10)	73	65.17	2	28.57
Piperacillin (100)	62	55.35	4	57.14
Amikacin (30)	78	69.64	5	71.42
Aztreonam (30)	65	58.63	3	42.85
Cefaperazone (75)	69	61.60	4	57.14
Ciprofloxacin (5)	50	44.64	3	42.85
Colistin (30)	112	100.0	7	100.0
Imipenem (10)	81	72.32	6	85.71
Piperacillintazobactam(100/10)	73	65.17	4	57.14

Antibiotic susceptibility pattern of *Acinetobacter baumanii* species

Acinetobacter species (n=77) were found to be 100% sensitive to colistin. *Among Acinetobacter* species 67.60% of *Acinetobacter baumannii* were found to be sensitive to imipenem followed by 64.78% sensitive for piperacillin-tazobactum and 61.97% for amikacin. Whereas maximum resistance in *Acinetobacter baumannii* was noted for ciprofloxacin and ampicillin sulbactam 73.24% and 69.02% respectively as shown in table-5.

All the strains of *Acinetobacter lwoffii* were sensitive to colistin 100% whereas the sensitivity of Acinetobacter lwoffii isolates for imipenem, piperacillin-tazobactam, amikacin and ceftriaxone were 66.66%, 66.66%, 50% and 66.66% respectively. Maximum resistance in *Acinetobacter lwoffii* was noted for ciprofloxacin 66.66% followed by ampicillin sulbactam 66.66%, cefaperazone 66.66% and gentamicin 50%.

TABLE-5: Antibiotic Sensitivity Pattern Of Acinetobacter Baumanii

Drugs (µg)	Acinetobacter baumanii (n=71)	
	S	%
Ampicillin-sulbactam (10/10)	22	30.98
Ceftazidime (30)	32	45.07
Ciprofloxacin (5)	19	26.76
Imipenem (10)	48	67.60
Gentamicin (10)	36	50.70
Amikacin (30)	44	61.97
Piperacillin-tazobactam (100/10)	46	64.78
Cefaperazone (75)	30	42.25
Ceftriaxone (30)	37	52.11
Piperacillin (100)	40	56.33
Colistin (30)	71	100.0

Antibiotic susceptibility pattern of isolates other than *Pseudomonas and Acinetobacter* species.

Two strains of *Burkholderia cepacia complex* were isolated both were found to be sensitive to colistin, trimethoprim-sulfamethoxazole. However both the isolates were 100% resistant for ciprofloxacin, imipenem.

Two strains of *Sphingomonas paucimobilis* were isolated both the strains were found to be sensitive to gentamicin, imipenem, ceftazidime, piperacillin and piperacillin-tazobactam .However both isolates were resistant to ciprofloxacin and amikacin.

It has been noted that overall all NFGNB showed high sensitivity to colistin 100%, imipenem 70.5%, amikacin 65.5% and piperacillin-tazobactam 64% however maximum resistance was noted for ciprofloxacin 61.5%.

DISCUSSION :

NFGNB that were considered to be contaminants in the past have now emerged as an important health care associated pathogen[1].The multidrug resistant organisms have increased over the time due to indiscriminate use of antibiotics which has led to increased duration of hospital stay and increased mortality and morbidity.

In the present study out of 1566 clinical samples 200 NFGNB were isolated accounting to isolation rate of (12.77%).Studies done by various workers have shown variable isolation rates of NFGNB, Mahajan R et al [9] reported 12.40% of NFGNB isolation rate. Juyal D et al [10]reported 9.32% isolation rate while studies done by Sharma D et al [11] reported 25.6% isolation rates which is higher as compared to other studies. The difference in isolation rate can be attributed to difference in population and living conditions of the study group.

In present study males 130 (65%) were more commonly affected with different infections caused by non-fermenter gram negative bacilli than females 70 (35%) as shown in table-1and graph-1, which is comparable with the studies done by Usha Rani P et al[12] which reported males (64%) as compared to females (36%). However result of the present study is in contrast with the study done by M. Zahid et al[102] where NFGNB infections were predominantly seen in females (53%) as compared to males (47%). This study is done in Pakistan so the higher incidence in females acquiring infections maybe attributed to their religious

beliefs of not following proper contraceptive practices therefore females are frequently admitted to hospitals for child birth. The present study has comparable results with the studies done by various workers. Male predominance maybe attributed to their more involvement in outdoor activities and therefore chances of exposure to infections are more likely as compared to females [3,14,15].

In the present study most common age group in both genders affected by NFGNB infections is 31-46 years (39%) followed by 15-30 years (27.5%). Least number of cases were seen in elderly patients who were more than 63 years of age (14.5%) as shown in table-1 and graph-1. In the study done by Usha Rani P et al[12] reported majority of patients were in the age group of 20-29 years (23.2%) followed by 30 - 39 years (20.8%). Least number of cases were in elderly patients in the age group of 70-79 years (4%). Benachinmardi K et al[3] reported equal prepondence in age group 21-30 years (20%) and 41-50 years (20%).

In the present study patients in the age group 31-46 years followed by 15-30 years are more commonly involved this may be due to more involvement to outdoor activities, more movement and have more active life style as compared to the elderly.

We have observe in our study that out of total 200 (12.77%) NFGNB isolated from various clinical samples, maximum NFGNB 87 (43.5%) were from Surgery department of which maximum 80 were from Surgery ward followed by Surgery ICU 06 and 01 from surgery OPD. Eighty two (41%) NFGNB were from Medicine department of which maximum 60 were from Medicine ward followed by Medicine ICU 17 and 05 from OPD while 31(15.5%) NFGNB were from Orthopedics department of which maximum 28 were from Orthopedics ward followed by 03 from orthopedics OPD as shown in table-2. Maniyan G et al [15] reported majority of NFGNB were isolated from surgical ward (40%) followed by ICU (20%). While Sharma D et al[11] reported maximum NFGNB isolates from burn ward (76.7%) followed by surgery ward (71%), surgery ICU (69.6%) and medicine ICU (68%).

In the present study total 200(12.77%) NFGNB isolates were processed from various clinical samples, maximum NFGNB were isolated from pus sample 95 (47.5%) followed by sputum 55 (27.5%), urine 23 (11.5%), blood 17 (8.5%), endotracheal secretion 07 (3.5%) and pleural fluid 03 (1.5%) as shown in graph-2.

Similar results were seen in study done by Bhatnagar R et al[16] in which maximum NFGNB isolates were from pus (49.20%) followed by sputum (19.84%). Malini A et al [17] also reported maximum NFGNB isolates from pus (61.90%) followed by sputum (12.69%). In the studies done by Maniyan G et al [15] maximum number of NFGNB were isolates were from pus (39%) followed by urine (18.1%).

Present studies showed maximum NFGNB are isolated from pus sample and sputum which coincides with the studies done by various workers that shows predominantly NFGNB are isolated from pus and respiratory samples.

In the present study *Pseudomonas aeruginosa* 112 (56%) were the commonest isolates followed by Acinetobacter baumanii 71 (35.5%). Other NFGNB which were isolated were Pseudomonas fluorescens- 7 isolates (3.5%), *Acinetobacter lwoffii*- 6 isolates (3%), *Burkholderia cepacia* complex -2 isolates (1%) and *Sphingomonas paucimobilis* - 2 isolates (1%) as shown in table-3 and graph-3.

Similar results were seen in the study done by Sharma D et al [11]where *Pseudomonas aeruginosa* and *Acinetobacter baumanii* isolated were (48.9%) and (33%) respectively. Mahajan R et al[9] reported *Pseudomonas aeruginosa* (54.54%) while *Acinetobacter baumanii* (41.08%). Malini A et al [18] reported Pseudomonas aeruginosa and *Acinetobacter baumanii* to be (53.8%) and (22.2%) respectively.

However studies done by Sharma M et al[18] have reported *Acinetobacter baumanii* to be most common isolate accounting to (50.4%) followed by *Pseudomonas aeruginosa*(38.1%). The present study is in concordance to the studies done by most of the workers. Where predominant NFGNB isolated were *Pseudomonas aeruginosa* followed by *Acinetobacter baumanii*.

Overall *Pseudomonas species* (n=119) showed maximum susceptibility to colistin (100%) followed by imipenem (73.10%) and amikacin (69.74%).In the present study *Pseudomonas aeruginosa* (n=112) showed maximum susceptibility to colistin (100%) followed by imipenem (72.32%), amikacin (69.64%) and ceftazidime (67.85%). While maximum resistance was observed for ciprofloxacin (55.34%), piperacillin(44.65%) and aztreonam (41.37%) table-4.

Study done by Sharma M et al [18] reported maximum susceptibility of *Pseudomonas aeruginosa* for colistin and imipenem (75.5%).Bhatnagar R et al [16] reported *Pseudomonas aeruginosa* maximum susceptibility for amikacin (85.5%) followed by imipenem (82.5%) and piperacillin (80%).Benachinmardi K et al [3] reported maximum susceptibility of *Pseudomonas aeruginosa* for amikacin (83.3%) followed by imipenem (80%) and piperacillin - tazobactam (73.3%).Maniyan G et al[15] reported susceptibility of *Pseudomonas aeruginosa* for colistin (100%) followed by imipenem (76.66%) and piperacillin-tazobactam (70%).

Overall *Acinetobacter species* (n=77) showed maximum susceptibility for colistin (100%) followed by imipenem (67.53%).In the present study *Acinetobacter baumanii* (n=71) showed maximum susceptibility for colistin (100%) followed by imipenem (67.6%), piperacillin-tazobactam (64.78%) and amikacin (61.97%) as shown in table-5.

Study done by Mahajan R et al [9] *Acinetobacter species* showed maximum susceptibility for colistin and imipenem (75.51%). Juyal D et al[10] reported *Acinetobacter* species showed maximum susceptibility for amikacin (75.47%) and imipenem (68.87%). Malini A et al [17] reported *Acinetobacter baumanii* with (100%) susceptibility for imipenem and (70%) susceptibility for piperacillin. Study done by Maniyal G et al [15] maximum susceptibility in *Acinetobacter baumanii* shown by imipenem (75%) followed by amikacin and piperacillin-tazobactam (72.2%).

6 strains of *Acinetobacter lwoffii* were isolated and found to be sensitive to colistin (100%), the isolates were found to be sensitive to imipenem(66.66%),piperacillin-tazobactam(66.66%), amikacin(50%) and ceftriaxone(66.66%).Maximum resistance in *Acinetobacter lwoffii* was noted for ciprofloxacin (66.66%), followed by ampicillin sulbactam(66.66%), cefaperazone(66.66%) and gentamicin(50%).

Study done by Maniyal G et al [15] showed high susceptibility in *Acinetobacter lwoffii* isolates for colistin (100%), imipenem (100%), Piperacillin-tazobactam (100%), amikacin (80%), ceftazidime(80%), cefotaxime (80%).

We have isolated two strains of *Burkholderia cepacia* complex which were found to be sensitive to colistin, trimethoprim-sulfamethoxazole. However both the isolates were resistant to ciprofloxacin and imipenem. In study done by Maniyal G et al [15] *Burkholderia cepacia* complex showed (100%) susceptibility to trimethoprim-sulfamethoxzole and imipenem/meropenem (66.7%).

We also report two strains of *Sphingomonas paucimobilis* which were found to be sensitive to gentamicin, imipenem, ceftazidime, piperacillin and piperacillin-tazobactam. However both isolates were resistant to ciprofloxacin and amikacin.

The worldwide resistance pattern of nosocomial pathogen shows wide variation from country to country over a period of time[11]. Proper identification and detection of antibiotic sensitivity pattern is of great value Therefore various international authorities have emphasized that every hospital should have antibiotic policy of its own [11]

It is importan documenting resistance among NFGNB as these strains often cause outbreak in intensive care unit settings and can limit therapeutic options due to high degree of multi drug resistance, leading to significant morbidity and mortality and has left us with few

therapeutic options. Evaluation of effective antibiotic options, judicious use of antibiotics and following hospital infection control measures like hand hygiene, use of personal protective equipment's, environmental cleaning, proper sterilization and disinfection practices for various instruments and devices used in patient care will help to fight against multi drug resistant NFGNB, which will help in effective management of patient.

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