

Original Research Article

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Isolation and Antibiogram of Nonfermentative Gram Negative Bacilli in various Clinical Specimens in a Tertiary Care Hospital, Jaipur, India

Jitendra¹, Shiv Kumar^{1*}, Sheetal Sharma², Jogender¹ and Suman Rishi¹

¹Department of Microbiology, NIMS Medical College, Jaipur, Rajasthan, India

²Department of Microbiology, Pacific Medical College, Udaipur, Rajasthan, India

*Corresponding author

ABSTRACT

Non Fermentative Gram Negative Bacilli were regarded to be Nonpathogenic and Contaminants in the past. Recently these bacilli became impending pathogenic with the ability to show resistance for commonly used antibiotics. These organisms isolated from clinical specimens with increasing frequency. So this study planned to isolate and antibiogram of Non-fermentative gram negative bacilli in various clinical specimens at tertiary care hospital at Jaipur. The present study was carried out on 49 of strains of Nonfermenters isolated from 397 non repetitive clinical samples like Pus, Wound discharge, Blood culture, Urine, Sputum, Ear discharge, Body fluid, ET Secretions and CSF etc. from all age's group. Conventional bacteriological methods were used for identification non fermenting gram negative bacilli and susceptibility testing was performed with the help of the Modified Kirby-Bauer disc diffusion method. This study comprises of 49 isolates of Non Fermentative Gram Negative Bacilli from total of 397 clinical specimens, collected from in- patients admitted to various departments during a period of 6 months. *Pseudomonas aeruginosa* was the most common isolate (57.14%) while *Acinetobacter baumannii* (24.48%) was the second most common isolated followed by *Pseudomonas fluorescens* (14.28%) and *Acinetobacter lwoffii* (4.08%). Among all clinical samples Pus yield maximum isolates of NFGNB i.e. 15(30.61%) followed by same account of Urine 8(16.32%) and Sputum 8(16.32%). Most sensitive drug against NFGNB were Polymyxin-B (100%) and Colistin (100%) followed by Imipenem (81.63%), Amikacin (71.42%). *Pseudomonas aeruginosa* and *Acinetobacter baumannii* have emerged as the most common Nonfermentative gram negative bacilli causing human infections. Alarming resistance rate was found towards commonly used antibiotics. Polymyxin B and Colistin showed excellent susceptibility against such bacteria followed by Imipenem and amikacin.

Keywords

Nonfermenters, Antibiogram, Modified Kirby-Bauer disc diffusion method, Nosocomial infections, Healthcare associated infections, Polymyxin-B, Colistin.

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Introduction

Nonfermenters are group of bacteria that are aerobic, non sporing, gram negative rod shaped bacilli and do not utilize carbohydrates as source of energy or degrade them by various metabolic pathways other than fermentation. These organisms although

frequently considered to be commensals or contaminants, but the pathogenic potential of these organisms has been established beyond doubt because of their frequent isolation from clinical specimens and their association with the disease (Koneman *et al.*, 2006).

We encounter many problems with these organisms because they are invariable resistance to common disinfectants like as chlorhexidine and quaternary ammonium compounds and now a day they account approximate 15% of all bacterial isolates of any clinical laboratory (Malini *et al.*, 2009).

Various organisms are present in these groups like *Pseudomonas*, *Acinetobacter*, *Alkaligenes*, *Burkholderia*, *Moraxella*, *Strenotrophomonas*, *Flavobacter*, *Oligella*, *Flavimonas* etc. (Meharwal *et al.*, 2002).

Pseudomonas aeruginosa is a common inhabitant of soil and moist environments (Minhas Akbar *et al.*, 2014; Ronald *et al.*, 1969). In hospitals, *Pseudomonas Aeruginosa* can be isolated from a variety of sources, including respiratory therapy equipments, antiseptics, soap, sinks, mops, medicines, physiotherapy equipment and hydrotherapy pools (Pollack *et al.*, 1995).

Data from more recent studies continue to show *Pseudomonas aeruginosa* as the second most common cause of nosocomial pneumonia, health care associated pneumonia and ventilator associated pneumonia (Gaynes *et al.*, 2005; Kollef *et al.*, 2005).

Acinetobacter species are ubiquitous in nature and have been found on or in soil, water, animals and humans. *Acinetobacter baumannii* is known to be recoverable from the skin, throat and rectum of humans and has been reported to be a healthcare-acquired colonizer of the respiratory tract. According to the Centres for Disease Control and Prevention (CDC), the species *A. baumannii* accounts for nearly 80% of reported *Acinetobacter* infections. This prolonged survival in the healthcare environment along with multidrug resistance, colonization potential, and contact transmission (hands, instruments, equipment) are some of the

challenging factors in *Acinetobacter* prevention and control (An APIC guide, 2010).

Members of the *genus Acinetobacter* usually invade compromised hosts and are seen in patients who have undergone endotracheal intubation, peritoneal dialysis, or central venous catheterization (Kiran Chawla *et al.*, 2013).

Pseudomonas aeruginosa and *Acinetobacter species* in particular are most often associated with carbapenem resistance. Carbapenem resistance appears to be due to metallo- β -Lactamase.

Excessive and indiscriminate used of broad spectrum antibiotics also responsible for antibiotic resistance (Mehta *et al.*, 2007). Patients become susceptible to non fermentive bacterial infections after abusive use of wide spectrum antimicrobial agents, prolonged treatment with immunosuppressive drugs, prolonged hospital stay, inadequate mechanical instruments like tracheostomy, catheterization, in case of burns, low birth weight babies or radiation (Gerard *et al.*, 1991).

The prevalence and rates of resistance in these organisms to available antimicrobials are increasing of particular concern is their resistance to β -lactams antibiotics, which is usually mediated by β -lactamase production. Widespread use of extended spectrum β -lactams antibiotics has resulted in the formation of potent enzymes that are able to inactivate many of the new generation of antimicrobial agents.

Extended spectrum β -lactamase (ESBL) are enzymes with the ability to hydrolyze the extended spectrum Cephalosporins, such as Cefotaxime, Ceftazidime and Cefoperazone. (Shahla Mansouri *et al.*, 2012).

Due to increasing frequency as isolation and increasing resistance against various antibiotics, it is important to know prevalence and antibiogram of Nonfermentive gram negative bacilli in hospital settings.

Materials and Methods

This Descriptive cross sectional study was carried out in the Bacteriology Department of Microbiology of National Institute of Medical Sciences (NIMS) University, Jaipur, District-Jaipur, Rajasthan, India for a period of six months (January 2017 to June 2017).

A total of 397 clinical specimens were found culture positive in the laboratory during the study period.

The organisms isolated and identified provisionally as NFGNB (n=49) were identified further by using a standard protocol for identification. (Koneman *et al.*, 2006).

All Non-repetitive isolates of NFGNB were collected from various clinical Specimens like Pus, Wound discharge, Blood culture, Urine, Sputum, Ear discharge, Body fluid, ET Secretions and CSF etc from Patients of all age group and both sex attending OPD, IPD, ICU and Wards in hospital, during the study period. Isolation of various isolates was confirmed by several of test like colony characters, morphology (Gram's stain), pigment production, motility (by hanging drop) and other biochemical tests like Catalase, Oxidase test, Indole, Methyl red, Voges Proskauer, Citrate utilization test, Urease test and Triple sugar iron Test, Oxidation/fermentation for glucose, lactose, xylose, mannitol and maltose (Hugh and Leifson's media), Lysine and Ornithine decarboxylase and Arginine dihydrolase activity, ONPG test, Esculin test etc. were done for isolation of the non-fermentative Gram negative bacilli.

Antimicrobial susceptibility

Antibiotic susceptibility testing was done by Modified Kirby Bauer's disc diffusion method as per CLSI guidelines 2017 using commercially available discs. *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as control strains.

Results and Discussion

A total 49 strains of nonfermenters were isolated from 397 various non-repetitive various clinical samples. Incidence of nonfermentative gram negative bacilli isolates from clinical samples was 12.34%.

Pseudomonas aeruginosa was the most common isolate (57.14%) while *Acinetobacter baumannii* (24.48%) was the second most common isolated followed by *Pseudomonas fluorescens* (14.28%) and *Acinetobacter lwoffii* (4.08%).

Out of total 397 samples 226 (56.92 %) samples from Male while 171 (43.07 %) from Female Patient.

Out of total 397 samples, maximum from age group between 21-30 years i.e. 23.17 % while minimum from <10 years of age i.e. 7.30 %.

The present had maximum Urine samples (38.03%), followed by Pus (21.66%), Sputum (10.07%), High vaginal swab (4.78%), ET secretion (4.28%), CSF (4.03%) Ear swab (3.77%), Pleural fluid (3.52%), Blood (2.77%), Catheter tip (01%), Semen (1%), Nasal swab (0.5%), Ascetic fluid (0.25%) and Bile fluid (0.25%) (Fig. 1).

Maximum samples of over study were sterile (44.33%). Gram Positive Cocci (25%) predominantly isolated in our study, followed by Gram negative bacilli other than NF GNB

(13%), NF GNB (12.34%), Normal flora (4%) and *Candida* species (2%) (Fig. 2).

Maximum Nonfermentive GNB isolated from Pus sample (15) followed by same amount of Urine (8) and Sputum (8), Ear swab (6), ET secretion (5), Blood (3) and (1) each from Catheter tip and Bile fluid (Table 1).

Out of 28 isolate of *Pseudomonas aeruginosa*, 25 (89.28%) isolates producing Blue- Green pigment (Pyocyanin), 1 (3.57%) was Non-pigmented, 2 (7.14%) isolates producing Brown Red pigment (Pyomelanin) (Fig. 3 and 4).

Antibiotic sensitivity testing results

Out of 49 isolates of NFGNB were processed for Antibiotic susceptibility testing was done by Modified Kirby Bauer's disc diffusion method as per CLSI guidelines 2017 using commercially available discs.

The antimicrobial sensitivity testing of isolated NFGNB as a pathogen showed that 100% of *P. aeruginosa* were sensitive to Polymyxin B and Colistin, 86% were sensitive to Imipenem, 75% were sensitive to Amikacin, 61% were sensitive to Ceftazidime-Clavulanate, 57% for Gentamicin and Piperacillin- Tazobactam, 53% for Cefepime, Ciprofloxacin and Meropenem. 50% sensitive to Ceftazidime and Aztreonam. 43% sensitive to Tobramycin. 39% sensitive to Cefotaxime. 36% sensitive to Ticarcillin and Piperacillin. 25% sensitive to Ceftriaxone 14% sensitive to Cotrimoxazole. 4% sensitive to Amoxycylav. (Table 2 and Fig. 5).

While the second most isolated NFGNB, *A. baumannii* showed highest sensitivity 100% sensitive to Polymyxin B and Colistin, 67% were sensitive to Imipenem, 59% were sensitive to Amikacin, 42% were sensitive to

Ceftazidime-Clavulanate, Aztreonam and Meropenem 34% for Gentamicin and Piperacillin- Tazobactam, Ceftriaxone, Tobramycin, Cotrimoxazole. 26% sensitive for Cefepime, Ceftazidime. 25% sensitive to Piperacillin. 17% sensitive to Ticarcillin, Ciprofloxacin and Cefotaxime, 8% sensitive to Amoxycylav.

While *Pseudomonas fluorescens* and *Acinetobacter lwoffii* showed 100% sensitivity to Polymyxin B and Colistin and total resistance to Amoxycylav.

Demographic profile of patients included in study

In our study higher samples were received in the age group of 21-30 years (23.17%) with over all preponderance of males (56.92%). This observations were compatible with study done by Jayapriya Sukumaran *et al.*, (2014).

Isolation of non- fermenters GNB

Among the diverse Clinical Specimens, in our study, the NFGNB were predominant in Pus samples (30.61%) followed by Sputum (16.32%) and Urine (16.32%). These result comparable with the study done by Kamalraj *et al.*, (2015), Dipak Bhargava *et al.*, (2015), Malini *et al.*, (2009). However, NFGNB predominantly reported in Ear Swabs (36%) by Aamal *et al.*, (2016).

Non fermenting Gram negative bacilli considered to be contaminants in the past have now emerged as important major pathogenic organisms. Previous studies reported varied isolation rates of NFGNB. In our study, isolation rate of NFGNB was 12.34%, which is in agreement with the study conducted by Hariom Sharan *et al.*, (2016) where the isolation rate was 13.82%. Our study is not in agreement with the studies

conducted by Sidhu *et al.*, (2010) (45.9%) and Vijaya *et al.*, (2000) 21.80% where very high isolation rate was reported. However, studies

conducted by Malini *et al.*, (2009) and Bruno *et al.*, (2011) showed very low isolation rate of 4.5% and 2.18% respectively.

Table.1 Distribution of NF GNB species in various clinical samples

NF GNB Species	Urine	Sputum	Pus	ET	Ear Swab	Blood	Catheter tip	Bile Fluid	Total (n=49)	Percentage (%)
<i>Pseudomonas aeruginosa</i>	3	4	9	3	6	1	1	1	28	57.14
<i>Acinetobacter baumannii</i>	3	2	3	2	-	2	-	-	12	24.48
<i>Pseudomonas fluorescens</i>	1	1	2	1	2	-	-	-	7	14.28
<i>Acinetobacter lwoffii</i>	1	-	1	-	-	-	-	-	2	4.08

Table.2 Species wise antibiotic susceptibility pattern of nonfermentative Gram negative bacilli

Name of the antibiotic	<i>Pseudomonas aeruginosa</i> (n=28)		<i>Acinetobacter baumannii</i> (n=12)		<i>Pseudomonas fluorescens</i> (n=7)		<i>Acinetobacter lwoffii</i> (n=2)	
	No. of isolates	%	No. of isolates	%	No. of isolates	%	No. of isolates	%
Amoxyclav	1	3.57	1	8.33	0	0	0	0
Ticarcillin	10	35.71	2	16.66	2	28.57	1	50
Piperacillin	10	35.71	3	25	2	28.57	1	50
Piperacillin-Tazobactam	16	57.14	4	33.33	3	42.85	2	100
Cefepime	15	53.57	3	26	2	28.57	1	50
Cefotaxime	11	39.28	2	16.66	2	28.57	0	0
Ceftriaxone	7	25	4	33.33	2	28.57	2	100
Ceftazidime	14	50	3	26	3	42.85	1	50
Ceftazidime-Clavulanate	17	60.71	5	41.66	4	57.14	2	100
Amikacin	21	75	7	58.33	6	85.71	1	50
Gentamicin	16	57.14	4	33.33	4	57.14	2	100
Tobramycin	12	42.85	4	33.33	2	28.57	0	0
Ciprofloxacin	15	53.57	2	16.66	3	42.85	0	0
Aztreonam	14	50	5	41.66	3	42.85	1	50
Imipenem	24	85.71	8	66.66	6	85.71	2	100
Meropenem	15	53.57	5	41.66	4	57.14	2	100
Cotrimoxazole	4	14.28	4	33.33	1	14.28	1	50
Polymyxin- B	28	100	12	100	7	100	2	100
Colistin	28	100	12	100	7	100	2	100

Fig. No.1: Distribution of Various Types of Clinical Samples (n=397)

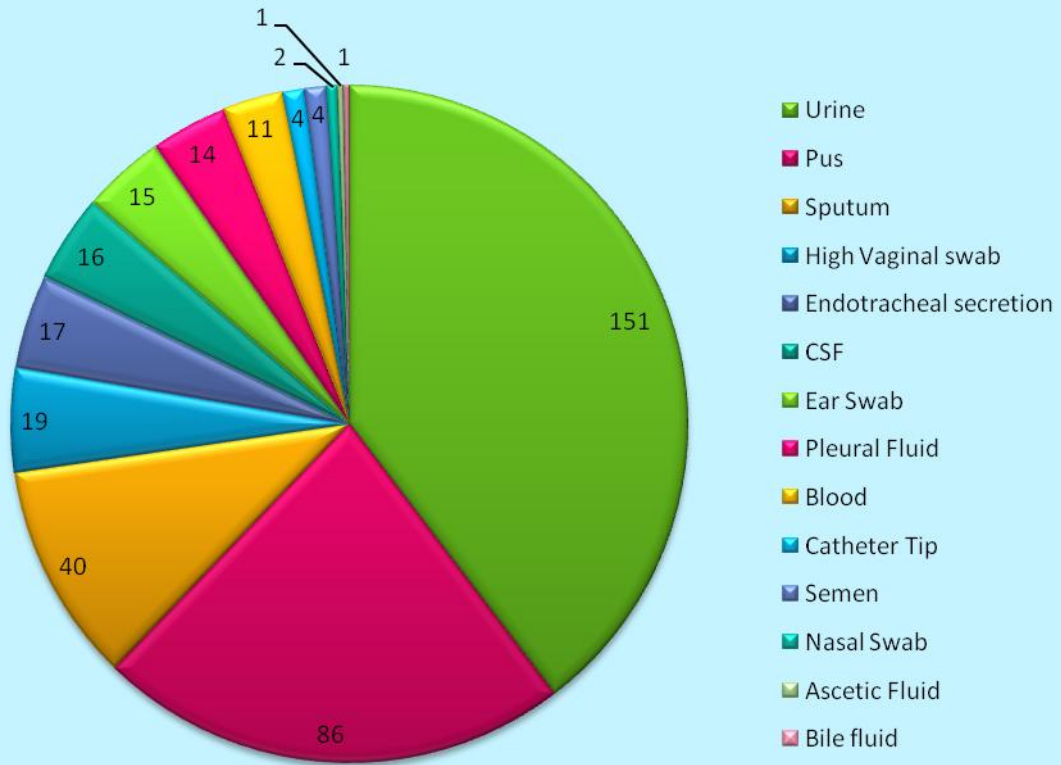


Fig. No. 2 : Isolation Rate of Various Organisms in Samples

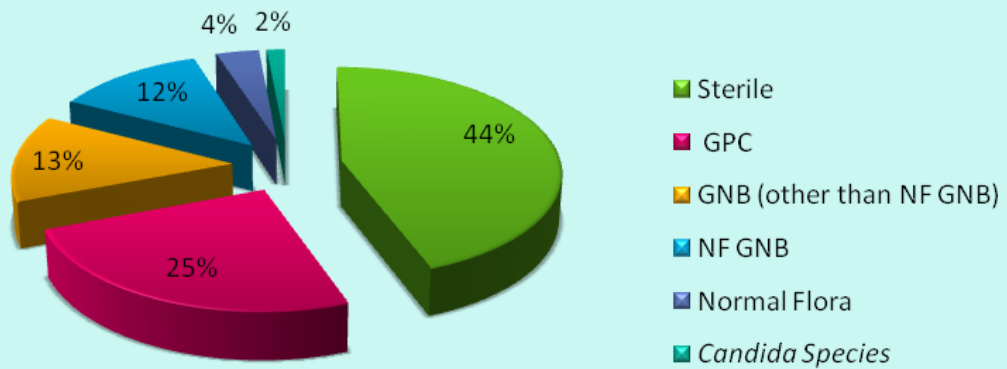


Fig. No. 3 : Percentage wise Distribution of Pigment producing *Pseudomonas aeruginosa*

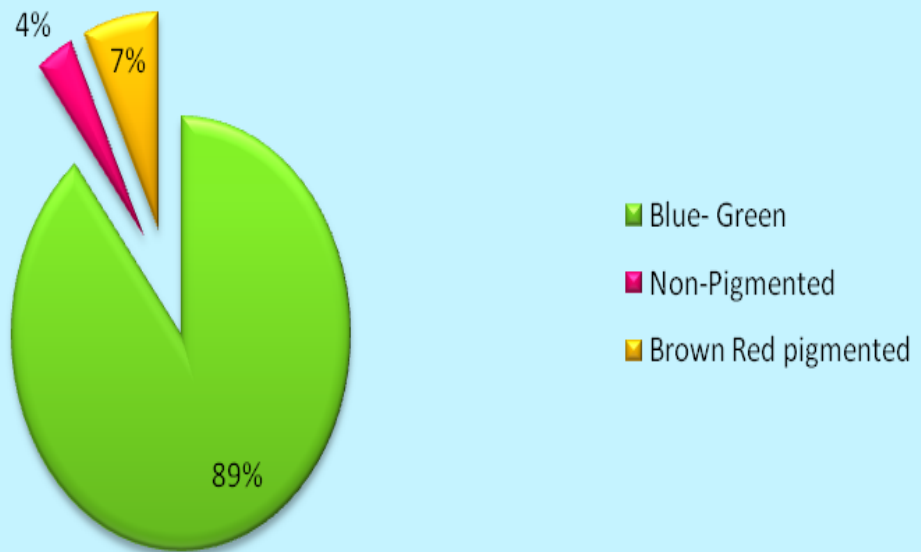
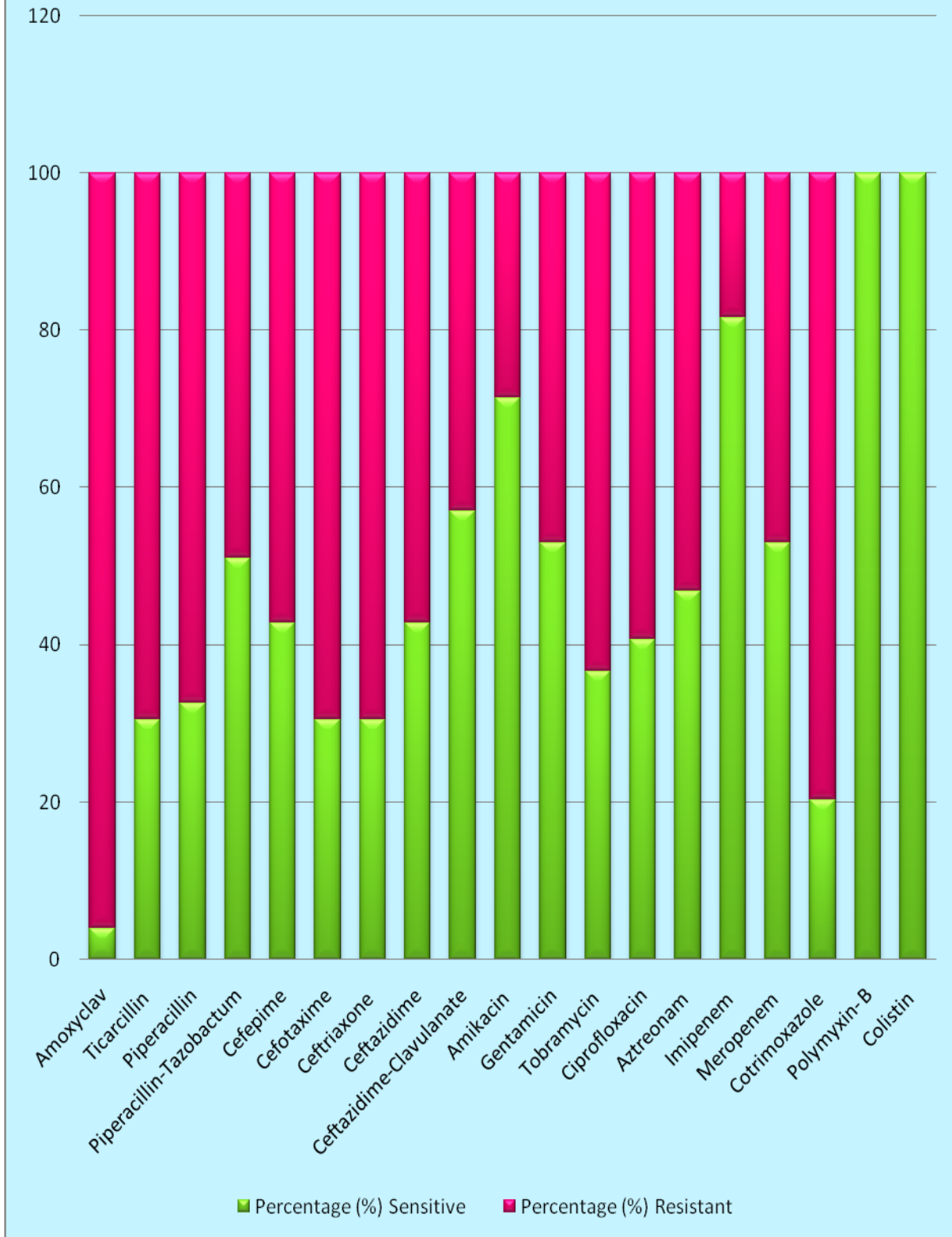


Fig.4 Antibiotic sensitivity pattern of *Pseudomonas aeruginosa* showing pyomelanin pigment



Fig. No. 5: Rate of Antibiotic Sensitivity Pattern in Nonfermentative Gram Negative Bacilli



Pseudomonas aeruginosa was found to be commonest nonfermenter in previous studies followed by *Acinetobacter baumannii* and this is in concordance to our results.

In the present study the most common NFGNB isolate was *Pseudomonas aeruginosa* 57.14%. This result has correlated well with the study conducted by Malini *et al.*, (2009) reported 64.4% while Arora *et al.*, (2010) reported 72.83%, Vijaya *et al.*, (2000) reported 78.94% while Juyal *et al.*, (2013) reported a low isolation rate of 38.21% *Pseudomonas aeruginosa*. The variation is likely due to number and type of clinical samples and geographical area of the study.

In the present study the second commonest isolate was *Acinetobacter baumannii* 24.48%. The result correlated well with the study conducted by Malini *et al.*, (2009) reported 25.3%. However, Juyal *et al.*, (2013) reported 29.27% in their study while Arora *et al.*, (2010) reported 8.4%. The variation is likely due to number and type of clinical samples and geographical area of the study.

Majority of *Pseudomonas aeruginosa* (32.14%) were isolated from pus specimens. This is comparable with the study conducted by Hariom Sharan *et al.*, (2016) who reported 26.32% isolation rate of *Pseudomonas aeruginosa* from Pus samples as dominant pathogen. However Dipak Bhargava *et al.*, (2015) reported 43.9% *Pseudomonas aeruginosa* from Pus samples.

In our study, majority of *Acinetobacter baumannii* were from Pus and Urine specimen (25% each). This is similar to study done by Kamalraj *et al.*, (2015)

However Sundaram Mohan *et al.*, (2016) reported 56.52%. *Acinetobacter baumannii* from Respiratory Specimens such as Endotracheal Tube and Sputum.

In Present Study, Isolation rate of *Pseudomonas fluorescens* was 14.28% and nearby isolation rate reported by Dipak Bhargava *et al.*, (2015). However Jayapriya Sukumaran *et al.*, (2014) reported only 2.6% *Pseudomonas species*.

Isolation rate of *Acinetobacter lwoffii* in our study was 4.08%. Similar isolation rate reported by Dipak Bhargava *et al.*, (2015) 3.6%.

Different types of pigments are produced by nonfermenters, which are helpful in making their species identification e.g. *Pseudomonas*, *Roseomonas*, *Stenotrophomonas maltophilia* etc. *Pseudomonas aeruginosa* produces Pyocyanin pigment in 90-96% of strains.

In our study out of 28 isolate of *Pseudomonas aeruginosa*, 25(89.28%) isolates producing blue green pigment (pyocyanin), similar observation was made in a study of Gokale *et al.*, (2012) that showed 94% of *Pseudomonas aeruginosa* isolates produced Pyocyanin pigment.

Antibiotic sensitivity and resistance of non fermenters GNB

In present study *Pseudomonas aeruginosa* showed 100% sensitivity to Polymyxin B and Colistin. This result is in concordance to study done by Hariom Sharan *et al.*, (2016). However Vikas Jain *et al.*, (2015) reported 71.43% sensitivity to Colistin and 78.57% sensitivity to Polymyxin B.

After Polymyxin B and Colistin *Pseudomonas aeruginosa* was highly sensitive to Imipenem (85.71%). It correlates well with the work of Kamalraj *et al.*, (2015) and Vikas Jain *et al.*, (2015) where sensitivity to imipenem was 88.4% and 78.57%, respectively. However, only 58.26% sensitivity reported against imipenem by Syed Hussain *et al.*, (2015).

Pseudomonas aeruginosa was 75% sensitive to Amikacin in our observation. Which is very similar to study done by Hariom Sharan *et al.*, (2016) where sensitivity to amikacin was 80%. But Vikas Jain *et al.*, (2015) reported only 50.00% sensitivity to Amikacin.

In present study, Very low sensitivity was present for Amoxyclav (3.57%) and Cotrimoxazole (14.28%). However Vikas Jain *et al.*, (2015) reported 50.00% sensitivity to Amoxyclav and Hariom Sharan *et al.*, (2016) reported 28% sensitivity to Cotrimoxazole.

Acinetobacter baumannii and *Pseudomonas aeruginosa* showed 100% sensitivity to Polymyxin B and Colistin. This result is in concordance to study done by Syed Hussain *et al.*, (2015). However Vikas Jain *et al.*, (2015) reported 80.00% sensitivity to both Colistin and Polymyxin B. In our study, *Acinetobacter baumannii* showed very low sensitivity against amoxyclav (8.33%), ticarcillin (16.66%), cefotaxime (16.66%) and ciprofloxacin (16.66%). Similar results had given by Hariom Sharan *et al.*, (2016) Sundaram Mohan *et al.*, (2016) and Syed Hussain *et al.*, (2015). However Kamalraj *et al.*, (2015) reported 66.66% sensitivity against Cefotaxime and 25% sensitivity to Amoxyclav.

In our study, other isolates were *Pseudomonas fluorescens* and *Acinetobacter lwoffii*. In them *Pseudomonas fluorescens* was 100% sensitivity to Polymyxin B and Colistin. This result is in concordance to study done by Hariom Sharan *et al.*, (2016). *Pseudomonas fluorescens* was totally resistant to Amoxyclav and had very low sensitivity against Cotrimoxazole. It correlates well with the work of Hariom Sharan *et al.*, (2016).

Acinetobacter lwoffii was very few to determine the antibiotic sensitivity. In present study over all Nonfermentive Gram Negative

Bacilli was highly sensitive to Polymyxin B and Colistin (100%). This result is in concordance to study done by Hariom Sharan *et al.*, (2016).

Nonfermentive Gram Negative Bacilli showed high resistance to Amoxyclav (95.91%) and Cotrimoxazole (79.59%) in present study. Our result is comparable to Hariom Sharan *et al.*, (2016) and Sundaram Mohan *et al.*, (2016) However Vikas Jain *et al.*, (2015) reported less resistance to Amoxyclav as (52.52%).

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