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Original article

Study on Non fermenting gram negative bacilli from various clinical samples in a tertiary care hospital.

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ABSTRACT

Non fermenting gram negative bacilli are a group of heterogenous, aerobic, non sporing bacteria. they are saprophyte in nature and are also found as commensals in man and animals. Hence this study aims at isolation, identification, and antibiotic susceptibility for non fermenting gram negative bacilli from clinical samples and to find out the clinical significance among the inpatient in meenakshi medical college and hospital kanchipuram. A total of 103 non fermenters isolated from clinical sample such as pus, sputum, urine, blood, and ear swab were included in the study. All these were identified by a battery of test as per standard laboratory technique. Aintimicrobial sensitivity testing was performed for all isolates by kirby bauer disc diffusion method. In these non fermenters, Pseudomonas were 83.5% followed by *Acinetobacter* 15.5% species was the second commonest non-fermenter followed by *shewanella sp.*,(0.9%). The NF GNBs are known cause of variety of nosocomial infection. Resistance pattern among the nosocomial bacterial pathogen may vary widely from place to place even within the same country over time. Thses nonfermenters are isolated from clinical specimens frequently and are resistant to most of the routinely used antibiotics showing that organisms need to be taken seriously and identified and not just regarded as contaminants.

1. Introduction

Non Fermenting gram negative bacteria constitutes about one-fifth of all GNB's. They are ubiquitous in nature, widely distributed although frequently considered as the commensal or contaminants but are frequently isolated from clinical specimens and are associated with disease[1]. Recently there has been a tremendous interest in these organisms as they are being isolated from clinical specimens with increasing frequency. Infections caused by other species are relatively infrequent[2]. In recent years due to the indiscriminate use of antimicrobials, NFGNB have emerged as important health care associated pathogens. They have been incriminated in infections such as bacteremia, meningitis, pneumonia, urinary tract infections, surgical site infections, wound infections, osteomyelitis, etc[3].

They are common in hospital settings and may be found on the surface of humidifiers, ventilator dialysis machines and other equipments as well as from the skin of hospital person. Recently they have been reported as the responsible for the serious infections such as septicemia and pneumonia. NF-GNB's are oxidase positive known for the ability to cause infections in debilitated and immunocompromised individuals[4]. NFGNB are known to account for 15% of all bacterial isolates from clinical microbiological laboratory. The organisms belonging to the genera Pseudomonas, Acinetobacter, Burkholdaria, Aeromonas, Achromobacter and Stenotrophomonas are normally present in air and on moist or dry surfaces[5] & therefore they have greater chances to infect patients in nosocomial setting.

They rarely cause serious infections in healthy persons and are infrequently identified as normal microbial floras in healthy individuals, but they are of greatest concern in hospitalized patients, particularly those in ICUs, where these opportunistic pathogens are capable of causing severe infections in critically ill and immunocompromised patients such as bacteremia, pneumonia, urinary tract infection, meningitis, endocarditis, burns, wound infection, eye infection, surgical site infection and osteomyelitis[1,5]. Pseudomonas spp.,having resistance to Carbapenem which is currently the most effective treatment option and the number of reported incidence are gradually increasing. Multidrug-resistant (MDR) strains of P. aeruginosa are often isolated among patients suffering from nosocomial infections, particularly those in the intensive care unit (ICU)[4,5].

Thus,infections caused by P. aeruginosa are particularly problematic because the organism is inherently resistant to many drug classes and is able to acquire resistance to all effective antimicrobial drugs. As an opportunistic infectious pathogen, P. aeruginosa can often lead to life-threatening diseases.The emergence in causing infection is of great concern to clinicians, spreading of resistance to commonly used antibiotics in both human populations has posed adverse impact on morbidity and mortality due to diseases caused by resistant bacteria[6]. The isolation rate of NF-GNB's were increasing in our lab, hence this

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study was undertaken to identify, speciate and to study the sensitivity pattern of NF-GNB's and also to know the clinical significance of this infected organisms.

2. Materials and methods:

The present study was undertaken at the department of microbiology, Meenakshi Medical College, Kanchipuram during the periods from Feb2013- Nov 2013 and a total of 103 Non-fermenters were isolated from clinical samples such as pus, sputum, urine and blood were included in the study. All collected specimens were processed by standard techniques. Organisms that failed to acidify the butts of triple sugar iron media were considered nonfermenters and subjected to a battery of tests like morphology, motility, oxidase, catalase, indole,urease, nitrate, citrate tests and oxidation-fermentation reactions of glucose, lactose, xylose, maltose, mannitol (Hugh-Leifson method). Antimicrobial susceptibility testing of all the isolates using Kirby-Bauer disc diffusion method and the results were interpreted as per clinical and laboratory standards institute (CLSI) recommendation.

3. Results:

Out of 103 NF isolated from pus, blood, sputum, urine were processed for a period of Feb2013-Nov2013.In these Pseudomonas spp., were maximum isolated followed by Acinetobacterspp.,. institute. All the cases with nonfermentative bacteraemia were immunosuppressed due to presence of one or the other underlying condition or disease.

Table- 1: Distribution of NFGNB from various clinical specimens

Isolate	Total no:	Percentage(%)
P.aeruginosa	86	83.5%
A.baumanii	13	12.7%
A.lwoffi	03	2.9%
Sh.putrefaciens	01	0.9%

Among the non-fermenters, Pseudomonas spp., were the maximum isolate followed by Acinetobacter spp.,.

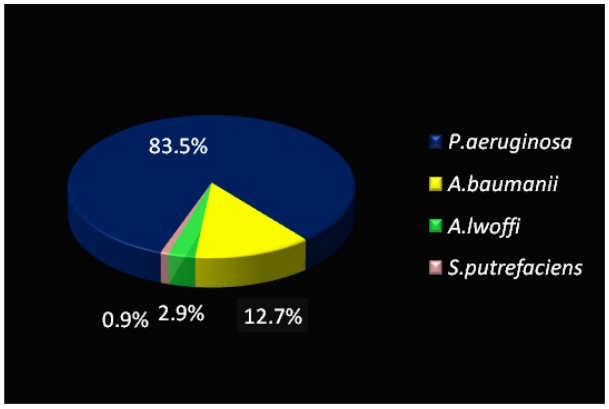


Table – 2: Distribution of different NFGNB various clinical specimens

Specimen	No. of cases	Percentage(%)
Pus	50	48.6%
Sputum	34	33%
urine	13	12.6%
Blood	06	5.8%

Among the non-fermenters, Pseudomonas spp., were the maximum isolate followed by Acinetobacter spp.,.

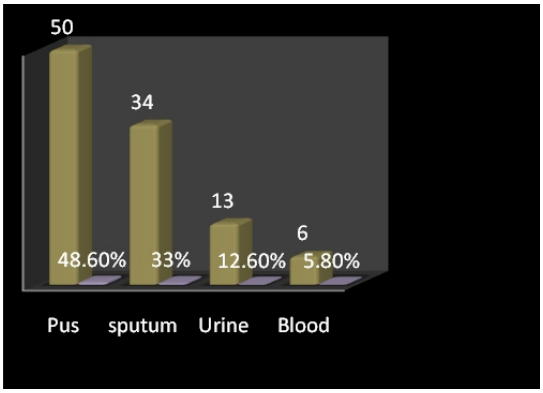


Table-3. Antibiotic resistance pattern of NFGNB against penicillin group

Antibiotics	*Number of Pseudomonas spp.,*	*Percentage(%)*	*Number of Acinetobacter spp.,*	*Percentage(%)*
Penicillin	61	75%	7	43.7%
Amoxyclav	40	46.5%	13	75%
Netilmicin	35	40.6%	8	50%
Piperacillin+tazo	28	32.5%	4	25%

Maximum resistance is seen to penicillin by Pseudomonas followed by Amoxyclav. In Acinetobacter spp., more resistance to Amoxyclav followed by Netilmicin.

Table-4Antibiotic resistance pattern of NFGNB to Cephalosporin group.

Antibiotics	*Number of Pseudomonas spp.,*	*Percentage(%)*	*Number of Acinetobacter spp.,*	*Percentage(%)*
Cefuroxime	48	55.6%	6	37.5%
Cefotaxime	37	43%	5	33.7%
Ceftriaxone	34	39.5%	7	43.7%
Cefepime	31	36%	7	43.7%
Cefaperazone	32	37.2%	4	25%
Ceftazidime	36	41.8%	6	37.2%

Maximum resistance is seen to Cefuroxime by Pseudomonas followed by Cefotaxime. In Acinetobacter more resistance to Ceftriaxone followed by Cefepime.

Table-5. Antibiotic resistance pattern of NFGNB to aminoglycoside

Antibiotics	*Number of Pseudomonas spp.,*	*Percentage(%)*	*Number of Acinetobacter spp.,*	*Percentage(%)*
Amikacin	42	48.8%	4	25%
Gentamicin	38	44.5%	5	31.2%

Maximum resistance is seen to Amikacin by Pseudomonas & Acinetobacter.

Table -6. Antibiotic resistance pattern of NFGNB to quinolones

Antibiotics	*Number of Pseudomonas spp.,*	*Percentage(%)*	*Number of Acinetobacter spp.,*	*Percentage(%)*
Ciprofloxacin	42	48.2%	5	31.2%
Ofloxacin	48	55.2%	03	18.7%

Maximum resistance is seen to Ciprofloxacin by Pseudomonas & Acinetobacter.

Table -7. Antibiotic resistance pattern of NFGNB to carbapenems groups

Antibiotics	*Number of Pseudomonas spp.,*	*Percentage%*	*Number of Acinetobacter spp.,*	*Percentage(%)*
Imipenem	10	11.6%	04	25%
Meropenem	09	10.4%	06	37.5%

Maximum resistance is seen to Imipenem by Pseudomonas. In Acinetobacter shows high resistance to Meropenem.

PHOTOGRAPHES

Figure: 1 Gram's stain showing gram negative bacilli of *P.aeruginosa.*

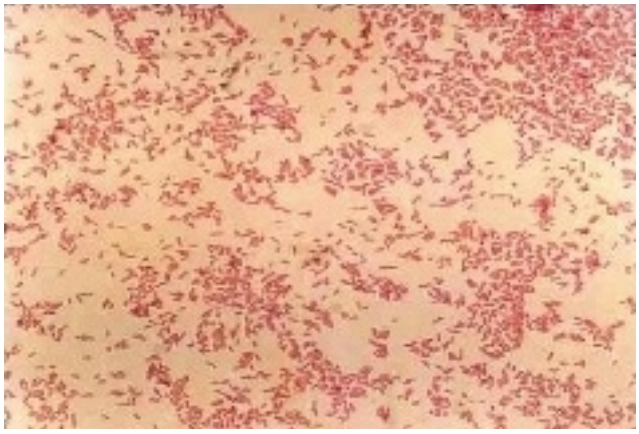


Figure: 2 Gram's stain showing gram negative Cocobacilli of *A.baumanii*

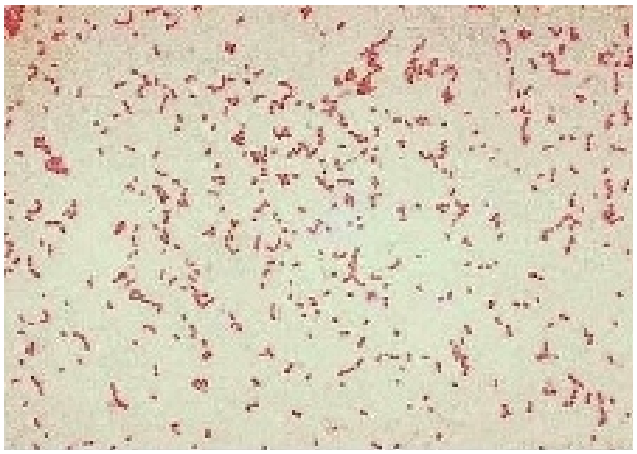


Figure: 3 Pigmented colonics of *p.aeruginosa* on Nutrient agar

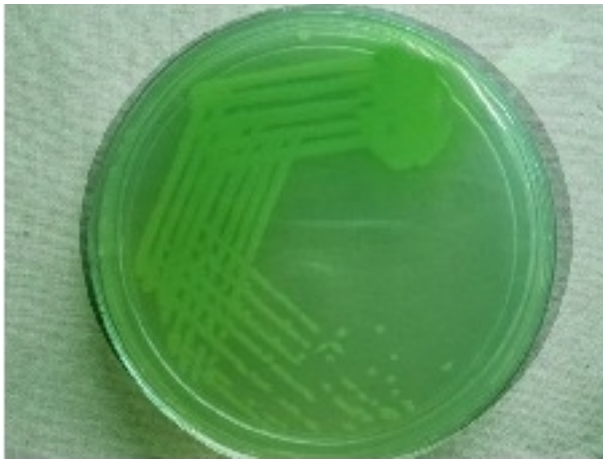


Figure: 4. *A. baumanii* on Blood agar

Figure. 5

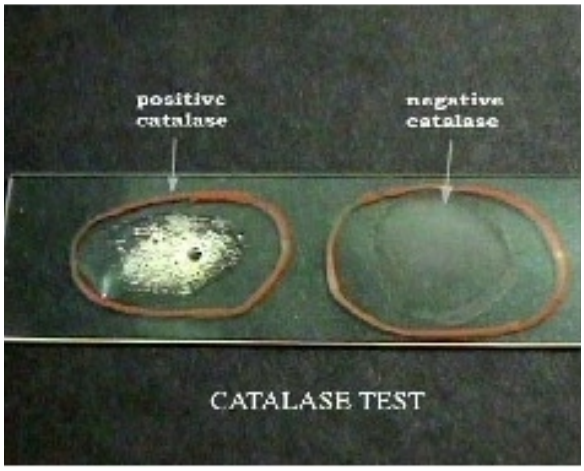
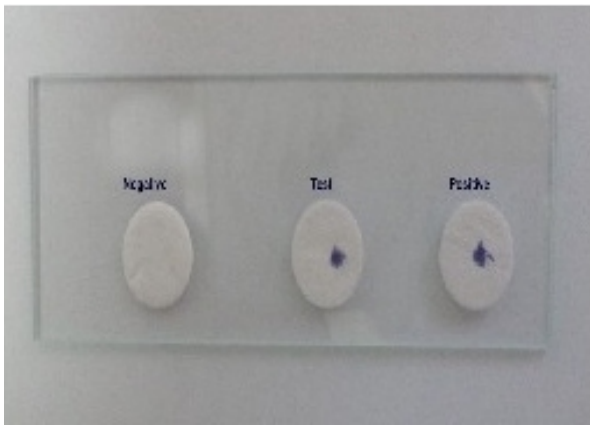


Figure. 6

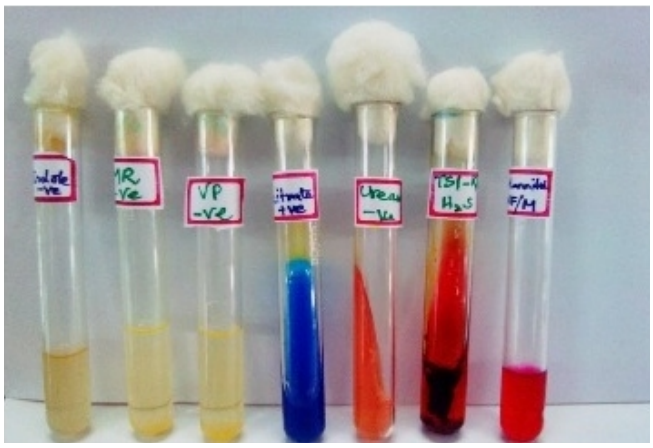


***Pseudomonas aeruginosa* shows Catalase and Oxidase test positive**

Figure. 7

***Pseudomoncis aeruginosa* biochemical reaction from L to R: Indole, MR, VP} Negative, Citrate Positive, Urease Negative, TSI - M/NC, Mannitor - NF/M, Nitrate test Positive.**

Figure. 8



Biochemical reactions of *shewanella putrefaciaens* from L to R: Indole, MR, VP Negative, Citrate Positive, TSI - K/K [H_2S Production]. Mannitol - NF/NM.

Figure. 9

***A.baumanii* biochemi calsreaditions from Lto R Indole, MR, VP Negative, Citrate positive, Urease Positive, TSI-K/K, Mannitor - NF/NM, Nitrate positive.**

Discusssion:

NF GNBs were considered as a contaminant in the past but now emerged as an important health care pathogen. Pseudomonas aeruginosa and Acinetobacter sp., are known to be nosocomial pathogen. In the present study out of 103 non fermenters, Pseudomonas were 83.5% followed by Acinetobacter 15.5% species was the second commonest non-fermenter followed by shewanella sp.,(0.9%). This is similar to studies done by(Malini and Vijaya et al 2009)[6,26]. Pseudomonas isolation was predominant in the study by done (Parimal et al 2013). In our study highest number of NF GNBs were from pus samples followed by sputum. This is similar to study done by Malini et al and Parimal et al (2013)[6]. In our study NF GNBs were isolated (18.4%) from various clinical samples.

The NF GNBs are known cause of variety of nosocomial infection (Larson EL1981)[8],(Bergogne.E 2001). Resistance pattern among the nosocomial bacterial pathogen may vary widely from place to place even within the same country over time (Prashanth K, Badrinath S. 2004)[7]. In our study out of 16

isolates of Acinetobacter species, revealed majority isolates as A.baumanii(81.25%) were as 3 isolates were found to be A.lwoffi(18.75%). Majority of the isolates were from pus specimen followed by urine. This is similar to study done by Parandekar P et al (2012).

Resistance to 3rd generation Cephalosporin, Cephotaxime showed (43%) and Ceftazidime showed (41%) in Pseudomonas. But it is higher than Angadi et al (2012) who reported (24.6%) to Cefotaxime and (25.6%) to Ceftazidime in Pseudomonas. Similarly Jayanthi et al (2012)[9] reported Cefotaxime resistance as (37%) and Ceftazidime resistance to (34%) for Acinetobacter which is concordant to our resistance of Acinetobacter as (37.2%) resistance to Ceftazidime and (33.7%) to Cefotaxime. Pseudomonas showed (44.8%) resistance to Gentamicin which is concordant with Murugan et al (2010)[10] who also reported (42.8%) resistance to Gentamicin, Jeya et al (2013) reported high resistance (51.6%) to Gentamicin.

In the present study Ciprofloxacin resistance to Pseudomonas is (48.2%) which is very much lower than Juyal et al (2013)[11] who was reported (73.7%) resistance to Ciprofloxacin for Pseudomonas. In our study (11.6%) of Imipenem resistance is reported to Pseudomanas which is concordant with Paul et al50 (9.8%) and Nicholas et al51 (9.8%) which is much higher resistant with Nagaveni et al[14](32%) and (71%) by Murugan[22]. in our present study (25%) were resistant to imipenem for Pseudomonas which is slightly lower than Juyal et al[11] who have been reported (31.3%) resistant to imipenem.

ESBL producing Pseudomonas aeruginosa were (38.3%) concordant with the study of Varun Goel et al (42.3%) and Agarwal et al[15](22.2%) where as Acinetobacter produces low prevalence of ESBLs as (25%). This similar study was concordant with Sinha et al[16] 28%). In the present study imipenem resistant strains of Pseudomonas aeruginosa were MBL producers, detected by phenotypic detection method of MBL production. In our study MBL producing strains of Pseudomonas were (11.6%) reasonably similar rate of MBL producers done by Nagaveni et al[17](24%) and (28%) by Anuradha et al[18] .

A total of 16 Acinetobacter spp. isolates were tested for the antibiotic susceptibility testing. Meropenem sensitivity of 90% has been reported in Shilpa .K Gokale et al55 study, & also well correlates with study conducted by Karlwosky et al[21](90%) and Taneja et a[20]7 12% of resistant which is concordant with our study(%). metallo beta lactamase was detected in 13% of the Meropenem resistant Acinetobacter baumannii isolates. Studies from the Indian subcontinent on the occurrence of metallo beta lactamase production by resistant Acinetobacter isolates are minimal.

An Indian study on the Acinetobacter baumanii species stated that 70.9% of these isolates produced Metallo beta lactamase (Uma KR et al 2009)[22], while another study reported from Kerala, India, states that 21% of the Acinetobacter baumannii isolates were found to be Metallo-β-lacatmase producers(Anil VK et al 2011)[23]. A large number of clinical isolates identified tentatively as Shewanella putrefaciens were shown to be S.algae by Nozue et al[24]. Most common infections of skin and soft tissue, and are usually associated with breaches in the skin such as ulcers or following trauma(Chen SC et al 1991)[25]. Among our isolate was found to be sputum sample.

According to the literature, most Shewanella infections are treated easily by a combination of surgical therapy, drainage and antibiotics. Poor outcome is associated most often with underlying disease. The main reason for this is that in most of the hospitals, all gram-negative and oxidase-positive organisms are repoted as Pseudomonas spp. And no further identification is done. A study done by Sharma et al who was also reported that Shewanella spp. were sensitivity to all antibiotics and which is similar to our study. Among the Non fermenters one case of Shewanella spp. was identified which was usually a environment organism and commensal[26].

Conclusion:

NFGNB though regarded as contaminants are important bacteria causing wide range of nosocomial infections. Variability in sensitivity pattern emphasizes the need for identification of NFGNB and to monitor their susceptibility patterns as it help in proper management of the infection caused by them. These organisms can also spread resistance to other susceptible bacteria by horizontal gene transfer. The most effective antibiotics are Amikacin, Imipenem, Ciprofloxacin, Ofloxacin, and Carbenicillin. Most of the NFs isolated were resistant to Penicillin group of drugs.

Repeated exposure of organisms to antimicrobial agents is thought to enhance the development and maintenance of resistance. Also presence of antimicrobial agent in sub lethal concentration makes an environment suitable for development of resistance.The sensitivity pattern changes from hospital to hospital and population to population. Often in clinical laboratory oxidase-positive non-fermenter gram-negative rods grown on routine laboratory culture media are considered as Pseudomonas spp. and further studied to exclude other oxidase-positive gram-negative rods that are thought to be rare. Shewanella spp. are very easy to identify and can easily grown on routine bacteriological media. So there is need to look for such rare organisms and not to dispose all oxidase-positive organisms as Pseudomonas. It may affect the overall outcome of the patients, but it will definitely help in better understanding of the epidemiology, pathogenesis and preventive aspects of such organisms.

Reference:

1) Koneman EW, Alen SD, janda WM, Schreckenbeiger PC, Winn WC. The Non fermenting gram negative Bacilli. In color Atlas and text book of diagnostic microbiology 5th edition, Philadelphia.J.B.Lippincott,1977;253-309.

2. Meherwal SK, Taneja N, Sharma SK, Sharma M. Complicated nosocomial UTI caused by nonfermenters. Indian J Urol 2002;18:123-8.

3. Fass RJ, Barnishan J, Solomon MC, Ayers LW. In vitro activities of quinolones, beta-lactams, tobramycin, and trimethoprim-sulfamethoxazole against nonfermentative gram-negative bacilli. Antimicrob Agents Chemother 1996;40:1412-8.

4. Ribeiro NFF, Heath CH, Kierath J, Rea S, Duncan-Smith M and Wood FM 2010 Burn wounds infected by contaminated water: case reports, review of the literature and recommendations for treatment. Burns 36 9–22.

5. Ali NH, Farooqui A, Khan A, Khan AY and Kazmi SU 2010 Microbial contamination of raw meat and its environment in retail shops in Karachi, Pakistan. J. Infect. Dev. Ctries. 4 382–388.

6. Malini A, Deepa EK, Prasad SR. Non fermenting Gram negative bacilli infections in a tertiary care hospital in Kolar, Karnataka J lab physicians 2009;1(2):62-6..

.7. Prashanth K, Badrinath S. In vitro susceptibility pattern of acinetobacter species to commonly used cephalosporins, quinolones, and aminoglycosides. Indian J Med Microbiol 2004;22:97-103.

8. Larson EL. Persistent carriage of Gram negative bacteria on hands. Am J Infect Control 1981;9:112-9.

9. Jayanthi S. Clinical distribution and antibiotic resistance pattern of non fermenting gram negative bacilli. (jan 2012).

10.Murugan S., Bakkiya Lakshmi R., Umadevi P. and Mani K.R. (2010) Int. J. of Microbiological Research, 1(30), 123-128.

11. Deepak Juyal, Rajat Prakash, Shamanth Prevalence of non-fermenting gram negative bacilli and their in vitro susceptibility pattern in a tertiary care hospital of Uttarakhand.(Dec 2013)

12.Paul D. Brown and Amicetus Izundu (2004) Pan. Am. J. Public Health, 16 (2):125-30.

13. Nicholas Ttroillet, Mathew H. Samore and Yehuda Carmeli (1997) Clinical Infectious Diseases, 25, 1094-8.

14. Agarwal KC, Antibiotic sensitivity test by disc diffusion . indian J Pathol Microbiol, 17:149-59(1994).

15. Nagaveni S., Rajeshwari H., Ajaykumar Oli, Patil S.A. and Kelmani Chandrakanth R. (2010) The Bioscan., (20), 251-253.

16. Anuradha K, Meena AK and Lakshmi V. Isolation of Burkholderia pseudomallei from a case of septicemia : A case report . Indian J Med Microbiology 2003; 21;129-132.

17.Shilpa K. Gokale. Characterisation and antibiotic susceptibility pattern of non fermenters. J.N.Medical college, Belgaum 2009.

18.Karlwosky JA , Draghi DC, Jones ME, Surviellance for antimicrobial susceptibility among clinical isolates of Pseudomonas and Acinetobacter from hospitalized patients in the united states, 1998 to 2001.

19. Taneja N, Maharwal S, Sharma M. Imipenem resistance in non-fermenters, which cause nosocomial urinary tract infections. Indian J Med Sci 2003;57: 294-99.

20.Uma KR, Srinivasa RR, Suchismita S, Shashikala P, Kanungo R, Jayachandran S, et al. Phenotypic and genotypic assays for detecting the prevalence of metallo-b-lactamases in the clinical isolates of Acinetobacter baumannii from a south Indian tertiary care hospital. J Med Microbiol. 2009; 58:430-35.

21.Sinha M,srinivasan H, Macaden R. Antibiotic resistance profile & extended beta lactamase (ESBL) production in Acinetobacter species. Indian Journal pathol microbial 2007; 126;63-67.

22.Anil VK, Vishnu SP, Kavitha R, Dinesh, Shamsul. The phenotypic detection of carbapenemase in the meropenem resistant Acinetobacter calcoaceticus–baumannii complex in a tertiary care hospital in south India. JCDR 2011; 5:223-26.

23.Vijaya BS, Govindan R, Preeti P, Kurt S, Daniel T, Prapas P, et al. The genetic relatedness and the molecular characterization of multi drug resistant Acinetobacter baumannii which was isolated in central Ohio, USA. Ann Clin Microbiol Antimicrobe. 2009; 8: 21-31.

24.Nozue H, Hayashi T, Hashimoto Y, Ezaki T, Hamaski K, Ohwada K, et al. Isolation and characterization of Shewanella alga from human clinical specimens and emendation of the description of S. alga Simidu et al, 1990, 335. Int J Syst Bacteriol 1992;42:628-34

25.Chen SC, Lawrence RH, Packham DR, Sorrell TC. Cellulitis due to Pseudomonas putrefaciens: possible production of exotoxins. Rev Infect Dis 1991;13:642-43.

26.Kasthuri Venkateswaran'lt Duane P. Moser,l Michael E. Dollhopf. Polyphasic taxonomy of the genus Shewanella and description of Shewanella oneidensis sp. Now International Journal of Systematic Bacteriology (1 999), 49,705-724.