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Prevalence of Non-Fermenting Gram Negative Bacilli from Clinical Isolates and their Antibiogram Profile

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A B S T R A C T

NFGNB also known as Non-fermenters (NFs) are emerging with increasing frequency as agents of opportunistic and often, serious infection as well as nosocomial infection. NFGNB are innately resistant to many antibiotics and are known to produce extended spectrum β-lactamases and metallo β-lactamases (MBL).Very few laboratories in India identify these organisms as routine as they are slow growing and requires special media and biochemical tests for identification. The rates of isolation of NFGNBs are increasing in Clinical Microbiology Laboratory, hence this study was done. To identify the Non – fermenters isolated from various clinical samples, to determine the antibiotic resistance pattern and To test the metallo β- lactamase activity of the isolated Non – fermenters. In This study comprises of 2758 samples of clinical specimen among 192 non-fermenting gram negative bacilli isolated from various clinical specimens like Respiratory tract samples, pus wound swab, urine and blood samples are taken as standard conventional methods. Non fermentative Gram negative bacilli (NFGNB) up to genus or species level were identified and processed by standard conventional method. Antibiotic susceptibility pattern of isolates was studied by Kirby Bauer Disc diffusion technique and resistant pattern also found. A total of 192 clinically significant culture isolates were obtained from different clinical samples using standard conventional methods. *Pseudomonas aeruginosa* was most common isolate (56.77%), followed by *Acinetobacter baumanii* (36.97 %), *A.lwoffii*(2.08%), *P. fluorescens* (4.16 %). Out of 49 Imipenem resistant organisms 22(44.89%) were Metalloβlactamase positive & 27 (55.10%) were Metalloβlactamase negative. They highly sensitive to Polymixin B and variable to carbapenamase group. The major risk factors for infection with non-fermenting gram negative bacilli infection were hospitalization of 5 day or more, surgical intervention and catheterization.

Introduction

Non-fermenting Gram-Negative bacilli (NFGNB) are a heterogeneous group of aerobic, nonsporing bacteria, which do not utilize glucose as source of energy or utilize it oxidatively. They comprise about 1/5th of all Gram Negative bacilli (GNB) and about 15%

of all bacterial isolates from a Clinical Microbiology Laboratory. [1, 2, 3]

They occur as saprophytes in the environment and some are also found as commensals in the human gut.[4] NFGNB also known as Non-fermenters (NFs) are emerging with increasing frequency as agents of opportunistic and often, serious infection as well as nosocomial infection.[1,2] They are most commonly isolated from patients with serious underlying disease, who have had prior antibiotic therapy, tracheostomy or endotracheal intubation, genitourinary instrumentation. [5]

They are frequently isolated from cases such as septicemia, meningitis, pneumonia, urinary tract infections & surgical wound infection. [1, 6] Among the species that are opportunistic pathogens in immunologically compromised host either by disease or treatment, *Pseudomonas aeruginosa (P.aeruginosa)* is eminent, followed by *Acinetobacter baumanii (A. baumanii), P.fluorescence, P.stutzeri, Stenotrophomonas maltophilia, P.putida, P.cepacia* [7]. Antimicrobial treatment of the infection caused by these agents is difficult due to its multidrug resistant (MDR) and rapid selection of high level MDR to various groups of antibiotics like β-lactam, Aminoglycosides and fluoroquinlones posing problem for both treatment and infection control.[8]

NFGNB are innately resistant to many antibiotics and are known to produce extended spectrum β-lactamases and metallo β-lactamases (MBL).[9,10] Very few laboratories in India identify these organisms as routine as they are slow growing and requires special media and biochemical tests for identification. The rates of isolation of NFGNBs are increasing in Clinical Microbiology Laboratory, hence this study was done.

The main and objectives of this study, to identify the Non –fermenters isolated from various clinical samples. And to determine the antibiotic resistance pattern of Non fermenters. Also to test the metallo β-lactamase activity of the isolated Non –fermenters.

Materials and Methods

This study comprises 192 non-fermenting gram negative bacilli clinically significant isolates which were collected from 2758 samples of clinical specimens, which was collected from IPD and OPD patients from the various departments of Surat Municipal Institute of Medical Education & Research (SMIMER), Surat during a period of 6 month from January 2015 to may 2015.

Collection and processing of Respiratory tract samples, Pus and wound swab/tissue, Urine and Blood by standard method. Samples were collected under aseptic precaution and transported to the lab.

The processing of sample was stricked and cultured on different media and incubated at 37º C for 24 hrs. We suspect the isolated GNB as a NFGNB if they show the following characteristics: 1) Lack of evidence for glucose fermentation and 2) Positive cytochrome oxidase reaction. Identification done by standard conventional method by biochemical reaction.

All the suspected NFGNBs were inoculated on Triple sugar iron agar medium (TSI). Organisms grew on Triple Sugar Iron and produced an alkaline reaction were provisionally considered to be non-fermenter gram negative bacilli, and were inoculated into Hugh and Leifson's medium for glucose, lactose, xylose and Mannitol to find out whether a particular organism was oxidizer or non-oxidizer and identified particular

organism its biochemical reactions characteristics.

The Non fermentative Gram negative bacilli (NFGNB) were identified up to genus or species level based on the following tests: Gram's Stain, Oxidase Test, Motility Test, Pigment Production, Indole Test, Urease Test, Citrate Test, Utilization of 10 % Lactose ,Catalase test, Triple Sugar Iorn, Oxidative fermentation (OF) of (Hugh-Leifson)-Glucose (G) Lactose (L), Mannitol (M), Xylose(X),Gelatin liquefaction,

Antibiotic susceptibility testing was done for all the NFGNBs isolates under the Standard CLSI guidelines by Kirby-Bauer's disc diffusion method. Isolated colonies (3-5) were picked up and emulsified in 2ml of nutrient broth and incubated at 37˚C for 2hours. Turbidity was compared and adjusted to 0.5 McFarland's tube/standard. Using a sterile cotton swab soaked with the broth, a lawn culture was made onto the dried surface of cation balanced Muller-Hinton agar (MHA).

Excess broth was expressed by rotating the swab against the inner side of the suspension tube. The plates were allowed to dry for 15 minutes. Later, pre-determined batteries of antimicrobial discs were dispensed onto the inoculated Muller-Hinton agar. Plates were incubated at 37˚C for 18-24 hours. Quality control of susceptibility testing was done by using *Pseudomonas aeruginosa* ATCC. The zone diameter were recorded and interpreted as sensitive, intermediate and resistant as per the CLSI zone interpretative criteria.

MBL-producing P. aeruginosa was suspected when the isolate was resistant to Imipenem. Screening and confirmation for the detection of MBL was done by disc potentiation test with EDTA-impregnated Imipenem discs[11]

Results and Discussion

In this present study, a total of 192 clinically significant culture isolates were obtained from different clinical samples using standard conventional methods. Of these 192 strains of *NFGNBs* isolated and identified, 62% were from males and 38% were from female patients. Majority of them were isolated from the patient age group of 21-40 year and followed by that of 41-60 year as shown in Chart 1.

Pseudomonas aeruginosa was most common isolate (56.77%), followed by *Acinetobacter baumanii* (36.97 %), *A. lwoffii* (2.08%), *P. fluorescens* (4.16 %).

Chart 2-(i) shows that Pseudomonas spp. were highly sensitive to Polymtxin B (total 109), Meropenem (total 102), Imipenem EDTA (total 100) followed by Ceftazidime (total 97), Cefepime (total 93) and Ceprofloxacin (total 93) while they were more resistent to Gentamicine (total 46), Levofloxacin (total 43) and Amikacin (total 27).

Chart 2 (iii) shows that Acinetobacter baumanii were more sensitive Polymyxine B (total 71) followed by Imipenem (total 52), Levofloxacin (total 51) and Cefepime (total 50) while they were more resistent to Cefuroxime (total 35), Cefoperazone (total 35) and Gentamicine (total 34)

Metalloβlactamase activity amongst NFGNBs In present study

Out of 49 Imipenem resistant organisms 22(44.89%) were Metalloβlactamase positive & 27 (55.10%) were Metalloβlactamase negative.

Non-fermentative gram-negative bacilli (non-fermenters) cause a significant number of infections, particularly in the hospitalized patients and immune-compromised hosts.

Pseudomonas aeruginosa and Acinetobacter baumanii are the most common non-fermenters pathogenic for humans. Infections caused by other species are relatively infrequent.[12] In the present study, 192 (14.1%) isolates were non-fermenting gram negative bacilli recovered from various clinical specimens. Similar observation has been made in present study which is shown in Table-4.

Table.1 No of isolates present in different wards

WARD	No Of Isolates
ICCU	15
SURGICAL WARD	56
MEDICINE WARD	20
ORTHO WARD	7
BURNS WARD	30
TB WARD	8
OPD	17
MICU	11
NICU	17
PICU	4
SICU	7

Table.2 Isolation rate of various NFGNBs from various clinical samples

Sr. No.	Organism/Specimen	Swab	Sputum	Fluid	BC	Pus	Total
1	*P. aeruginosa*	75	12	2	10	10	109 (56.77%)
2	*A. baumannii*	44	6	4	12	5	71 (36.97%)
3	*P. fluorescens*	6	1	1	0	0	8 (4.16%)
5	*A. lwoffii*	3	0	0	1	0	4 (2.08%)
Total		128 (66.66%)	19 (9.89%)	7 (3.64%)	23 (11.97%)	15 (7.81%)	192

Chart.1 Age and gender wise distribution of clinical isolate of *NFGNBs*

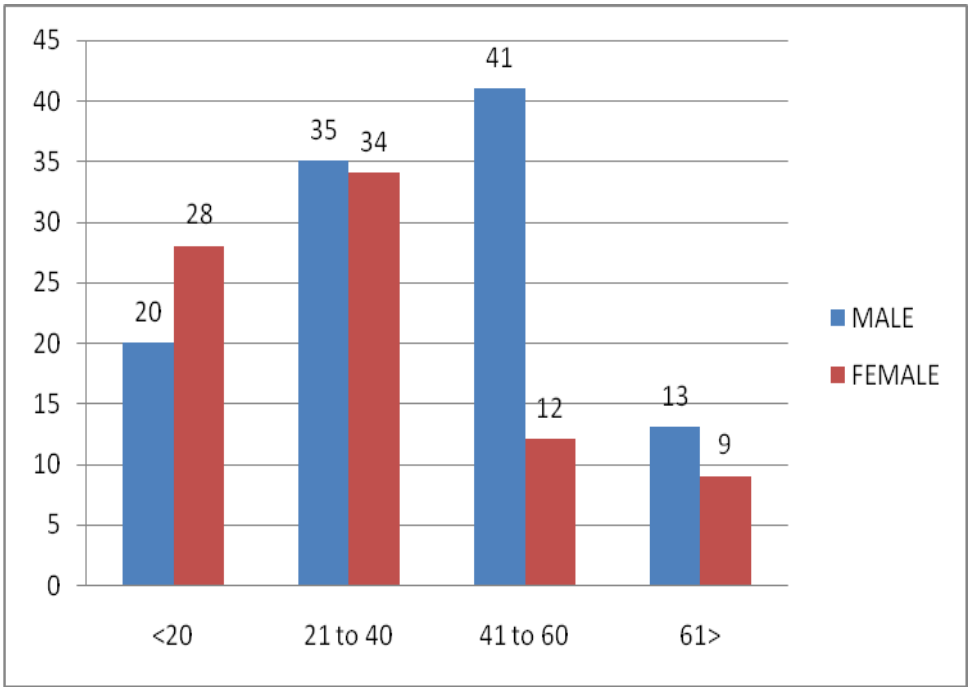

Chart.2 Sensitivity pattern of NFGNB isolated in present study; (Sensitivity pattern of *P.aeruginosa*, P.fleuroscence, *A.baumannii* and *A.lwoffii* discussed here)

Chart.2(i) Antibiotic sensitivity of *P.aeruginosa*

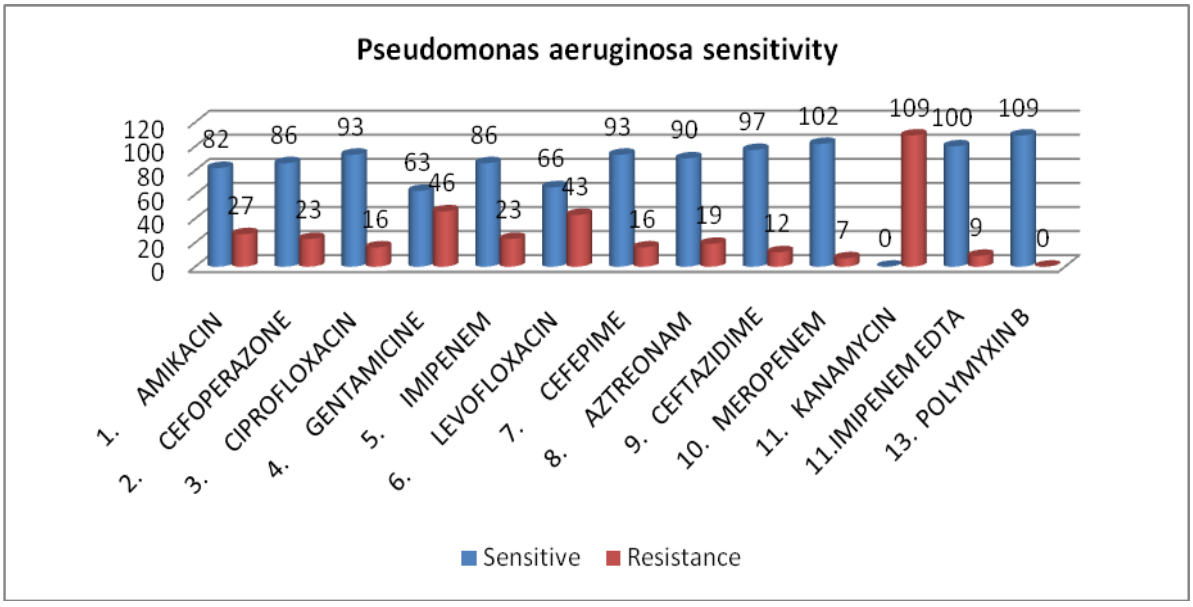

Chart.2(ii) *P. fluorescence* sensitivity

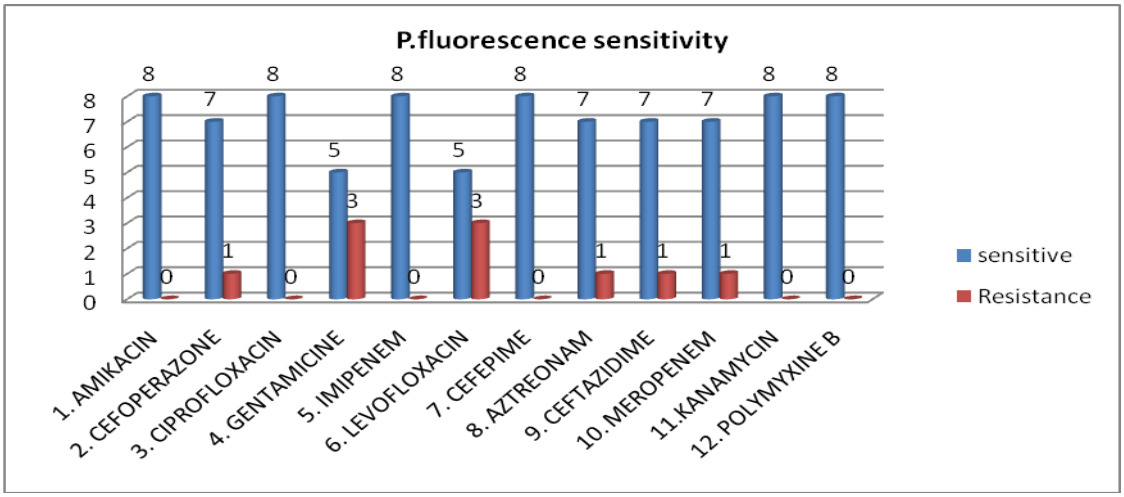

Chart.2(iii) *A. baumanii* sensitivity

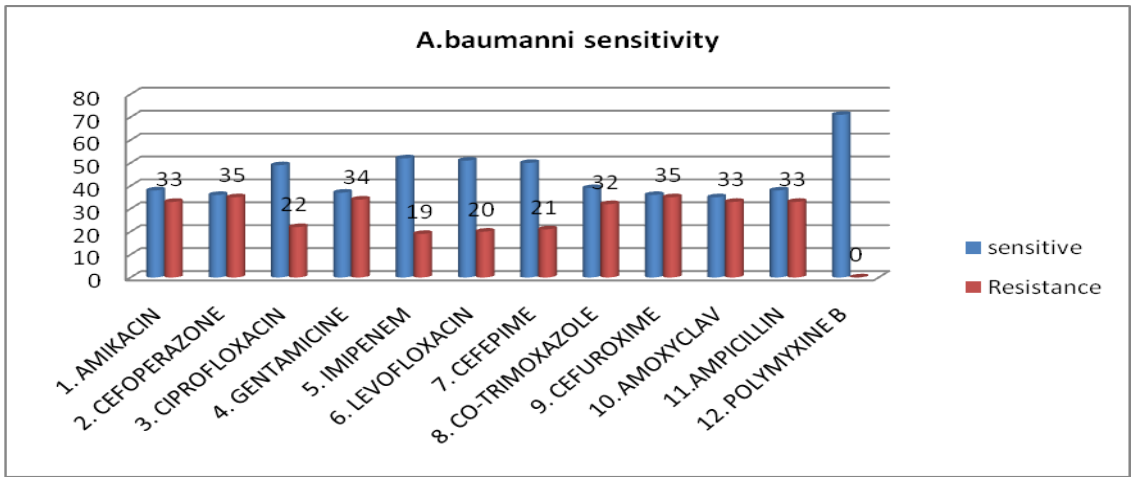

Chart.2(iv) *A.lwoffii* sensitivity

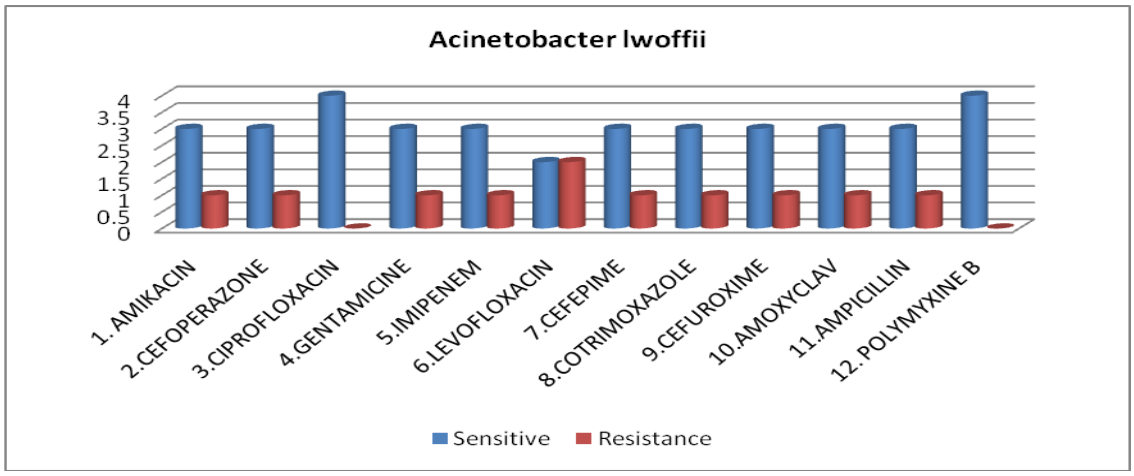

Table.3 Antimicrobial sensitivity pattern

Antibiotic tested	*Pseudomonas aeruginosa*		*Psedomonas fleuoroscence*		*Acinetobacter baumanii*		*Acinetobacter lwoffi*	
	S	**R**	**S**	**R**	**S**	**R**	**S**	**R**
Imipenem	86	23	8	0	52	19	3	1
Gentamicin	63	46	5	3	37	34	3	1
Amikacin	82	27	8	0	38	33	3	1
Amoxyclav	0	0	0	0	35	6	3	1
Ciprofloxacin	93	16	8	0	49	22	4	0
Ceftazidime	97	12	7	1	0	0	0	0
Cefepime	93	16	8	0	49	22	3	1
Cefoperazone	86	23	7	1	36	35	3	1
Polymyxin b	109	0	8	0	71	0	4	0
Cotrimoxazole	0	0	0	0	38	33	3	1
Imipenem-EDTA	100	9	7	1	0	0	0	0
Meropenem	102	7	7	1	0	0	0	0
Aztreonam	90	19	7	1	0	0	0	0
Cefuroxime	0	0	0	0	33	38	3	1
Ampicilin	0	0	0	0	33	38	3	1
Levofloxacin	66	43	5	3	50	21	2	2
Kanamycin	0	109	8	0	0	0	0	0

Table.4 Comparison of species wise distribution of NFGNBs in various studies

Species	Present study n=192	Malini *et al.*, [13] n=193
P.aeruginosa	109 (56.7%)	104 (53.8%)
A.baumanii	71 (36.97%)	43 (22.2%)
P.fluorescence	8 (4.16%)	21 (10.8%)
Sphingobacterium species	0	10 (5.02%)
A.lwoffii	4 (2.08%)	6 (3.1%)
S.maltophilia	0	5 (2.6%)
A.faecalis	0	1 (0.5%)
Others*	0	*3 (1.5%)

Out of which 109 (56.7%) were *Pseudomonas aeruginosa*, 71 (36.97%) were *Acinetobacter baumanii* complex, 8 (4.16%) were *Pseudomonas fluorescence*, 4 (2.08%) were *Acinetobacter lwoffii*. Study conducted by Malini *et al.*, reported nonfermenting gram negative bacilli isolation rate as 4.5%. *Pseudomonas aeruginosa* as the most common isolate (53.8%).[13]

Maximum number of non fermenting gram negative bacilli were isolated from Swab (66.66%) followed by Blood (11.97%), Sputum (9.89%), Pus (7.81%) and Fluid (3.64%).

In present study there was preponderance of the NFGNBs infections in males as compared to females. Similar observation is made in other studies also. [14, 15]

Studies done by Mishra *et al*., [2], Kitch *et al*., [12]& Yashodhara *et al*., [16] have shown that NFGNBs have been most commonly isolated from pus/swab sample, which is similar to present study.

In present study *P.aeruginosa* 109 (56.77%) & *A.baumanii* 71 (36.97%) were the most common isolates amongst all organisms causing local infections like cellulitis, diabetic foot, burns etc. which is similar to other studies done by Rajan *et al*., & Wisplinghoff *et al*., [14, 15]
P.aeruginosa (56.77%) was the main etiological agent responsible for local infections in present study, however in studies done by Yashodhara *et al.*, [16], Mishra *et al.*, [2], Resmi Rajan *et al.*, [14] and Cristane *et al.*, [17]it was respectively 66.95%, 66%, 89.9% & 72.5%.

Infection at burns site is because of injury associated with breakdown of normal skin, immune defects and selection of antibiotics with inadequate coverage for this pathogen. [18]

Four cultures in study done by Malini *et al.*, [13] showed mixed (two NFGNBs) infections while, we haven't isolated any mixed NFGNB infections.

In conclusion, a prospective study conducted to know the prevalence of different beta lactamase among, 192 non-fermenting gram negative bacilli clinically significant isolates which were collected from various clinical samples and cultured using standard conventional methods. Of these 192 strains, 109 (56.77%) were from males and 83 (43.22%) were female. Most of them belonged to the age group 21-40 year and followed by patient of 41-60. Clinical isolates of *Pseudomonas aeruginosa*, maximum isolates 128 (66%) are isolated from swab followed by 19 (9%) from sputum and 7(3%) from fluid, 23 (11 %) from blood and 15 (7 %) from pus and other samples.

Most of samples were collected from surgical wards, followed by burn ward, medical ward, TB wards, orthopaedic ward, NICU, MICU, OPD PICU and ICUU. Maximum resistant isolates of *Pseudomonas aeruginosa* were isolated from swab samples.

Of these 109 (56.77%) Pseudomonas aeruginosa spp, 8 (4.16) were pseudomonas fluorescence, 71 (36.97%) were Acinetobacter baumanii and 4 (2.08%) were Acinetobacter lwoffii. All the isolates of Pseudomonas aeruginosa were sensitive to Polymixin B and least resistance was observed towards Meropenem. All the isolates of Acinetobacter were sensitive to Polymyxin B and least resisnce to Amoxyclav. All the strains of Acinetobacter lwoffii also sensitive to imipenem. Out of 49 Imipenem resistant organisms 22 (44.89%) were Metalloβlactamase positive & 27 (55.10%) were Metalloβlactamase negative. The major risk factors for infection with non-fermenting gram negative bacilli infection were hospitalization of 5 day or more, surgical intervention and catheterization.

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