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Frequency of non-fermenting Gram Negative Bacilli in ICU setting: Experience at a tertiary care center in Karachi

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Abstract:

Objective: To determine the frequency of Non-fermenting Gram Negative Bacilli (NFGNB) from the clinical specimens obtained from patients admitted in intensive care units (ICUs).

Material and Methods: This study was conducted at the Microbiology Department of the Basic Medical Sciences Institute (BMSI), Jinnah Postgraduate Medical Centre (JPMC), Karachi. In all, 200 specimens were collected from different ICUs of surgical and chest wards from August 2015 to August 2016. All patients admitted in the ICUs for more than 48 hours were included in the study. The NFGNB were identified by the standard micro-biological laboratory tests.

Results: Age group 41-60 years was more vulnerable with a second peak after the age of 60 years. There were 108(54%) males and 92(46%) females. Out of 156 culture positive cases, 52(33.33%) were NFGNB i.e. Pseudomonas species, while 104(66.67%) were other bacteria. Highest rate of culture positivity was seen in pus (84%), followed by respiratory secretions (82%), urine (66. 6%), and blood samples (60%). Respiratory secretions were the most common samples from which NFGNB were isolated, i.e. 36 (44%). Distribution of bacterial pathogens in different wards showed 36 cases of Pseudomonas aeruginosa from surgical and 16 from chest wards, respectively, followed by Klesiella pneumoniae, 26 cases from surgical and 14 from chest wards. Regarding E-coli, 18 cases were isolated from surgical wards and 4 cases from chest ward.

Conclusion: In conclusion, patients in ICUs are commonly affected by NFGNB. Pseudomonas aeruginosa was the most common nosocomial microorganism isolated from this cohort of patients.

Keywords: Pseudomonas aeruginosa, Non-fermenting Gram Negative Bacilli, respiratory secretions, Klesiella pneumoniae, E-coli, Acinetobacter species

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Introduction:

Non-fastidious, aerobic, gram negative bacteria are common pathogens in humans. Conventionally, these micro organisms have been sub-divided into two major groups: fermentative gram negative bacteria (fermenters) and non-fermentative gram negative bacteria (non-fermenters). The dividing line between these types of bacteria was based more on convention rather than on well-defined genetic or phenotypic characteristics.[1]

Non-fermentative gram negative bacteria (NFGNB) are strict aerobes i.e., they drive their energy only by oxidation of sugars rather than by fermentation. Because they do not ferment glucose, they are called non-fermenters, in contrast to the members of the enterobacteriaceae, which ferment glucose.[2]

Non-fermenting gram negative bacilli are ubiquitous micro-organisms that could be isolated from soil, water, plants and animals, including humans. These organisms have a predilection for moist environment and are often found in the hospital around therapeutic equipment

which is exposed to water as well as common utility areas such as sinks, bath tubs and house keeping, cleaning material.[3] These are primarily opportunistic bacteria, causing infections mainly in severely ill and immuno-compromised patients. Many of these organisms have become problematic in the hospital in part due to their ability to survive in various habitats including aqueous, moist environment (e.g. Pseudomonas species) or dry surfaces (Acinetobacter species).[4] Infections are either of endogenous or exogenous origin, depending on several factors such as: use of immunosuppressant substances, use of wide spectrum antimicrobial agents and prolonged surgical procedures.[5]

Bloodstream infections (BSIs), which represent a failure of the immune system to contain infection at a focal site and consequent disseminated disease, are a major cause of morbidity and mortality. It is estimated that hospitalized patients in the United States have 250,000 episodes of nosocomial BSIs annually, and when these infections occur in patients in intensive care units (ICUs), they are associated with a significant mortality rate of 35%, 24 additional hospital days, and excess hospital costs of $40,000 per survivor.[6-13]

Non-fermenting bacteraemia affects patients of both sexes equally, and patients of all ages, mostly older people, and those with underlying diseases or conditions, such as transplant patients, carcinoma patients and patients on steroid treatment. On the other hand, community cases occur mainly in patients with non-malignant underlying diseases such as diabetes mellitus, renal failure or human immunodeficiency virus-I (HIV-1) infection.[14]

Although Gram-positive bacteria are the most common causes of BSIs, gram-negative bacteremia carries higher risks of severe sepsis, septic shock and death.[1] Patients undergoing haemodialysis have a compromised immune system that made them susceptible to a number of infectious diseases, requiring frequent hospitalization and surgery, which increases the possibility of nosocomial infections.[15]

NFGNB, normally saprophytes, cause serious infections in immuno-compromised and hospitalized patients especially those admitted in ICUs. These organisms further worsen the situation by virtue of their multi-drug resistance and thus limit therapeutic options. NFGNB are very important as these strains often cause outbreaks in the ICU setting. These organisms are potentially dangerous in the ICU setting and lead to increased financial burden for patients. They might also spread resistance to other susceptible bacteria by horizontal gene transfer.[16]

No socomially acquired infection is defined as infection that occurs more than 48 hours after hospital admission, infection that occurs less than 48 hours after admission to hospital in patients who had been hospitalized within the 2 weeks prior to admission, or infection that occurs more than 48 hours after hospital admission in patients who have been transferred from an outside hospital or nursing home.[17]

Pseudomonas aeruginosa is a leading cause of nosocomial infections and the most frequent organism isolated from specimens. In addition, NFGNB also differ in terms of pathogenic potential and transmissibility. Identification to species level is required for proper clinical management of patients.[4]

This study was conducted to determine the frequency of isolation of NFGNB from the clinical specimens obtained from intensive care units (ICUs) of surgical and chest ward patients of a large public tertiary care center.

Materials and Methods:

This study was conducted in the Microbiology Department, Basic Medical Sciences Institute (BMSI), Jinnah Postgraduate Medical Centre (JPMC), Karachi, Pakistan. In all, 200 clinical specimens were collected from different ICUs of surgical and chest wards of the hospital, from August 2015 to August 2016. Patients admitted for at least 48 hours in various ICUs/surgical wards with chronic illnesses irrespective of gender and age were included in the study. These included immuno-compromised patients, using

Table-1: Surgical site infection (n=183)

Age group (Years)	Number	Percent
15-20	23	11.5
21-30	16	8.0
31-40	30	15.0
41-50	41	20.5
51-60	44	22.0
> 60	46	23.0
Total	200	100.0

Gender	Number	Percent
Male	108	54.0
Female	92	46.0
Total	200	100.0

Table-2: Distribution of total micro-organisms isolated on the basis of different wards (n=156)

Organisms	Ward		Total	%
	Surgical ICU	Chest ICU		
Pseudomonas species	36	16	52	33.33%
Candida albican	05	03	08	5.12%
Citrobacter	02	0	02	1.28%
CoNS (Coaugulase negative Staph.)	04	01	05	3.2%
Escherichia coli	18	04	22	14.1%
Klebsiella species	26	14	40	25.6%
Proteus species	13	01	14	8.97%
Staphylococcus aureus	09	02	11	7.05%
Staphylococcus saprophyticus	01	01	02	1.28%
Total	114	42	156	100%

Table-3: Distribution of non-fermenting gram negative bacteria (NFGNB) isolates from specimens of different sites (n=52)

	Respiratory secretions	Pus	Urine	Blood	Total
NFGNB	36 (69.27%)	15 (28.8%)	01 (1.92%)	0 (0%)	52

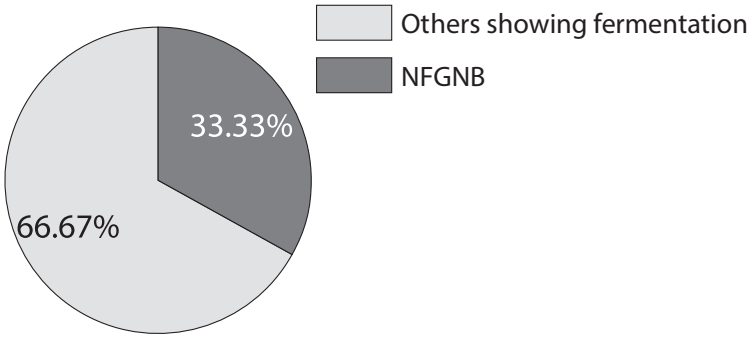

Figure-1: Distribution of total isolated micro-organisms (n=156)

immuno-suppressive substances, patients with history of broad spectrum anti-microbial agents, patients with prolonged surgical procedures and mechanical instrumentation, severe sepsis and septic shock. Patients admitted in ICUs for less than 48 hours and not transferred from any other hospital were excluded. The research was conducted in accordance with the tenets of Declaration of Helsinki in 1975, as revised in 2008.

All the specimens were inoculated on Blood agar and MacConkey agar medium. The plates were incubated at 35° C for 24-48 hours and examined for the growth. Gram staining of all the growths was done. Gram negative bacilli were identified by set of following classical biochemical tests such as: motility test by hanging drop method, fermentation tests of various sugars like glucose, lactose, sucrose, mannitol, xylose, maltose and triple sugar iron (TSI) agar, Simon citrate, Indole, and Urease production etc.

The NFGNBs were identified by using following battery of tests: colonial morphology, pigment production, odour, oxidase test, oxidation fermentation test, reactions in TSI agar, indole, methyl red, Voges Proskauer test and citrate (IMVIC) and motility tests.[18]

The data were entered and analyzed by SPSS version 13.0. Only descriptive statistics were used, mainly in the form of frequencies and percentages.

Results:

Out of 200 samples, 100(50%) were respiratory secretions, 50(25%) pus from wounds of patients, 30(15%) urine from catheterized patients and 20(10%) blood samples.

The frequency of infection was directly related to age; 11.5% in age group 15-20 years, 15% in 31-40 years, 20.5% in age group 41-50 years, 22% and 23% in age groups 51-60 years and more than 60 years, respectively. Males were more susceptible than females with male to female ratio of 1.2:1 (Table 1).

Figure 1 shows different groups of microorgan-

isms isolated in different samples. Out of 156 culture positive cases, 52(33.33%) were positive for NFGNB i.e. Pseudomonas species, while 104(66.67%) cases were positive for other bacteria.

Most common organism isolated in specimens was pseudomonas species in 52(33.33%) samples and majority of them were isolated from surgical ICUs (36 out of 52 specimens); however, 16 were positive in ICU of respiratory ailments. Among the other isolates, Klebsiella species were the commonest organisms isolated from 40(25.6%) specimens; 26 of them were isolated from surgical ICU and remaining 14 from ICU of respiratory diseases. E-coli was isolated in 22 of 156 culture positive samples; 18 were isolated from surgical ICUs and 4 from ICU of chest ward. Proteus mirabilis was isolated in 14 samples with 13 from surgical ICU. Staphylococcus aureus was isolated in 11 specimens with 9 from surgical ICU (Table-2).

In Table-3, distribution of NFGNB in various samples is shown. Highest rate of culture positivity was seen in respiratory secretions; 36 of 52 cases, second common was pus (15 cases), and no NFGNB was isolated from blood cultures.

Discussion:

Hospital acquired infections are a major cause of morbidity and mortality in patients admitted in the ICUs. These patients have 5-10 fold greater risk of developing nosocomial infections with aerobic gram negative bacilli when compared with those admitted in general hospital wards. These higher rates of infection are mostly related to factors such as underlying disease, duration of stay in the ICU, number and types of invasive procedures performed, site of infection and association with nosocomial multidrug resistant pathogens. ICUs receive a large number of patients irrespective of their diagnosis, age and disease status; so there is strong likelihood of badly infected patients coming in contact with immuno-compromised patients. The inflow of critical patients makes ICU environment and equipments a harbor for the various pathogenic organisms capable of infecting in epidemic form. Nosocomial lower respiratory tract infections, especially pneumonia, are common in ICU patients and in accordance with this, most of the isolates were obtained from respiratory tracts.

NFGNB are very important as these strains often cause outbreaks in the ICU setting; conversely, there are limited therapeutic options due to a higher prevalence of multi-drug resistance. This means that these organisms are potentially dangerous in the ICU setting and could lead to increased financial burden for patients.[16-20]

In this study of 200 patients, organisms were isolated in 156(78%) cases, while remaining 44(22%) cases showed no bacterial growth. Of 156 positive cases, 52 were NFGNB i.e. Pseudomonas species.

In our study, as the age of patients increased, the incidence of infection also increased, suggesting immuno-compromised status of patients with chronic underlying diseases. Young patients also showed growth of NFGNB in acute, poly-trauma traumas.

Male and female patients both are affected in ICUs. In our study, out of total 52 cases of NFGNB, female cases were 28(53.8%) and male patients were 24(46.2%). This is quite concordant with the study of Vidal et al., in which, NFGNB caused bacteremia and affected patients of both sexes and all ages, mostly older patients and with underlying disease.[14] Relatively higher number of old patients involved in this study revealed the lower resistance in older patients and slight higher rate of infection in females also reflected lower resistance.

According to the type of specimens, the majority of positive cultures for NFGNB were seen in respiratory secretions; 36 of 52 cases, while growth was seen in 15 pus samples out of 52 cases. Only one positive culture was obtained from urine samples and no NFGNB was isolated from blood samples. Thus, common sites of recovery of organisms in the present study were respiratory secretions, followed by pus and urine. A study conducted by Frota and Moreira on 1,834 cases

found NFGNB i.e. Pseudomonas aeruginosa as the most frequent species (69.98%).[5] This study was in agreement with the present study but only showed difference regarding the site of isolation of NFGNB. In the present study, majority of organisms were grown in respiratory secretions (69.2%) and pus (28.8%), while Frota and Moreira indicated the change in pattern of positivity in which urine was the specimen affected more frequently than respiratory secretions.[5]

In a study from Pakistan by Zafar et al. on 100 isolates from patients admitted in ICUs, only 7% of NFGNB mainly Pseudomonas species were isolated. Acinetobacter species was the commonest NFGNB found in the above study.[21]

In another study from Pakistan by Izhar et al. conducted on 62 ICU patients, the sites of isolation were mainly from respiratory secretions(54%), blood(33%), urine(3%) and others (10%).[22] The isolates obtained were identified as Pseudomonas species (including Pseudomonas aeruginosa) (34%) followed by other gram negative rods; this study was also in accordance with our study.

In another study by Kucukates and Kocazeybek, majority of micro-organisms were isolated from respiratory tract (50.3%) and blood (39.3%).[23] Pseudomonas species was the most frequently isolated gram negative bacteria (32.7%). This study is in agreement to our data, in which respiratory tract was main site of infection and Pseudomonas species were the main isolate.

In another study from India conducted by Kumari et al., analysis of respiratory tract specimens of 370 ICU patients was done. Specimens of 274(74%) patients were culture positive, remaining showed normal flora and no microorganism was isolated in 96(26%) cases.[24] Most common gram negative isolates in order of frequency were: NFGNB (31.9%), Pseudomonas aeruginosa (21.5%), remaining were Gram negative rods including Klebsiella, Escherichia coli, Proteus mirabilis, Citrobacter and other gram negative rods. The highest number of gram negative bacilli (GNB) isolates were from those above 50 years of age (24.8%) followed by 31-40 years (19.2%) and those between 21-30 years (15.9%) which is concordant to this study.

The findings of a study by Bataineh and Alrashed were very much comparable to our study.[25] According to them, out of 100 gram negative bacteria isolated from different sites collected from patients admitted in ICU, the most common isolate was Pseudomonas aeruginosa (21%) followed by gram negative rods like Klebsiella(20%), and E.coli(15%). Most common sites of isolation were respiratory tract(65%), urine(14%), wound(11%), and blood(7%). Pseudomonas aeruginosa was the most frequent isolate from respiratory specimens.[25]

Lockhart et al. in their study of 74,394 cases in ICU patients during 12 years period, found Pseudomonas aeruginosa in 22.2% cases, and most of the specimens belonged to respiratory tract (52.1%), as in the present study.[26]

Conclusions:
In conclusion, Pseudomonas aeruginosa was the most common non-fermenting gram negative bacilli found in our study. Respiratory secretions were the most common specimens affected by this organism.

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Role and contribution of authors:
Dr Pushpa Valiram, collected the data and wrote the initial write-up.

Dr Suresh Kumar, review critically and made final changes.

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