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Original Research Article

Study of Gram Negative Non Fermenting Bacilli from Surgical Site Infections in a Tertiary Care Hospital in Kolkata

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ABSTRACT

Surgical site infections (SSI) result in increase in hospital stay and strain on hospital economy. Increasing number of Non-fermentative Gram-negative bacilli (NFGNB) isolates from SSI which are frequently multi drug resistant (MDR), necessitate proper identification and susceptibility pattern for effective management. The aim of this study was to determine the point prevalence of SSI by NFGNBs and to assess their susceptibility pattern to antimicrobials. A prospective study of 300 cases of SSI was studied from pus samples received in the Department of Microbiology, N.R.S.M.C.H over a period of six months (Jan 2018 to June 2018). These clinical samples were subjected to classical bacteriological diagnostics and NFGNB were identified using a standard protocol. Antimicrobial susceptibility testing was done by Kirby Bauer disc diffusion method. Out of 300 SSI cases, significantly 89 (29.6%) isolates were NFGNB. Genus and species wise predominant isolates were *Pseudomonas aeruginosa* (52.8%) and *Acinetobacter baumannii* (39.3%); others were *Stenotrophomonas maltophilia* (3.37%), *Acinetobacter lwoffii* (2.24%), *Pseudomonas fluorescence* (1.12%) and *Sphingomonas paucimobilis* (1.12%). All *Acinetobacter baumannii* isolates could multiply at 10ºC which is a significant collateral finding. Imipenem resistance was noted in 82% *Acinetobacter baumannii* and 9% *Pseudomonas aeruginosa*, whereas 76% *Acinetobacter baumannii* and 5% *Pseudomonas aeruginosa* were resistant to meropenem. Colistin resistance was found in 2.85% *Acinetobacter baumannii* isolates. All 3 *Stenotrophomonas maltophilia* were resistant to Levofloxacin and Cotrimoxazole. Our study showed high number of MDR NFGNB causing SSI. As mostly NFGNB infections are exogenous, this study highlights importance of following strict aseptic measures to control such infections.

Keywords: NFGNB, SSI

INTRODUCTION

Surgical site infections (SSI) are ones which occur within 30 days post surgery where surgery took place. [1-2] The Centers for Disease Control and Prevention (CDC) [1] classifies SSI into three major categories: i) Superficial infections, which are localized to skin and subcutaneous tissue, characterized locally by redness, pain, warmth and swelling and are resolved by local incision and discharge of pus. ii) Deep incisional infections, affecting muscles and fascia with presence of abscess, which require surgical excision of deep wound edges and iii) infection of abdominal organs or anatomical spaces, which require

surgical procedures in locations other than the initial incision site.

Abdominal surgical wound infections in adults are defined as infections that occur within maximum 30 days post abdominal surgery and contribute to increased post-surgical morbidity and mortality. Most common complications are abscesses, wound infections and necroses. [3] According to CDC, SSI is one of the most common healthcare-associated infections (HAI) and account for $3.2 billion in attributable cost per year in acute care hospitals. [4]

A recent prevalence study found that SSI was the most common HAI accounting for 31% of all HAI among hospitalized patients. [5] SSI are associated with increased morbidity and mortality rates, accounting for additional annual hospital charges of ~$1.6 billion in the United States alone. [6]

NFGNB are a taxonomically diverse group of aerobic, nonsporing Gram negative bacilli that either do not utilize glucose as a source of energy or utilize it oxidatively. [7] They occur as saprophytes in the environment and some are also found as commensals in human gut. [7-8] In recent years due to liberal and empirical use of antibiotics, NFGNB have emerged as important health care associated pathogens. They have been incriminated in infections such as septicemia, pneumonia, urinary tract infections and surgical site infections [9] and are being isolated from various clinical specimens. Although frequently considered as contaminants, their pathogenic potential has been proven beyond doubt by their frequent isolation from clinical samples and their association with disease, more so in immunocompromised patients. NFGNB associated with different nosocomial infections including SSI are becoming increasingly resistant to commonly used antimicrobial agents. [10-11] They exhibit resistance not only to the beta lactam and other group of antibiotics but also to carbapenems recently. [11]

There are very few studies from India wherein the various NFGNB, isolated from SSI cases, have been identified and their clinical significance assessed. Hence, this study was undertaken to identify various NFGNB isolated from patients with SSI from our hospital, a tertiary care hospital at Kolkata. The study was also done to assess their clinical significance and antimicrobial susceptibility pattern.

Aims and Objectives:

The present study aims to isolate and characterize the NFGNBs in patients of SSI, determine their susceptibility pattern and assess their clinical significance.

MATERIALS AND METHODS

The present study was undertaken in the department of Microbiology, N.R.S.M.C.H, Kolkata for a period of six months from January 2018 to June 2018 after approval from the Institutional Ethics Committee. Three hundred patients of SSI included in the study were selected on the citeria as- non healing wound as revealed on 6^{th} day post surgery along with discharge from the wound, redness and pain around the surgical site and leucocytosis. Duplicate pus samples from each SSI site were collected using sterile inoculating loop or with a sterile swab stick or aspirating with a sterile syringe and needle where possible. These samples were immediately processed for Gram stain and aerobic culture on nutrient agar, blood agar and Mac Conkey agar media and incubated at 37°C for 18-24 hours.

Identification exercise of the isolates involves study of colony morphology, Gram stained morphology and motility, and ability to grow at 10^0C and 42^0C. Further biochemical tests undertaken were ability to ferment glucose, sucrose. maltose in Hugh Leison's OF media, oxidase, catalase, urease and indole production, ability to grow on Simon's citrate slant, H_2S production in TSI agar, amino acid utilisatison pattern. Finally genus and species level identification were determined

following the interpretative scheme of Mac Faddin *et al* (1976) [12] Forbes B *et al* [13] and Winn W Jr *et al* (2006). [14]
Further the identification was confirmed by Automated identification system-Vitek2

The clinical significance of the NFGNB isolated was assessed retrospectively by analyzing the case sheets for a combination of relevant laboratory and clinical criteria. The clinical criteria included presence of features like fever, signs of redness and pain around the area where surgery was done and drainage of cloudy fluid from the surgical wound site. [1] Laboratory criteria included:-presence of pus cells along with Gram negative bacilli or Gram negative coccobacilli in the stained smear directly from the sample, monomicrobial infection, isolation of the same organism from both the samples and leucocytosis.

Antibiotic susceptibility was done using commercially available discs (HiMedia) of ampicillin (10mcg), levofloxacin (5mcg), amikacin (10mcg), gentamicin (10mcg), cefotaxime (30mcg), cefepime (30mcg), piperacillin-tazobactam (100/10 mcg), cotrimoxazole (25mcg), ceftazidime (30mcg) cefoperazone-sulbactam, imipenem (10mcg), meropenem (10mcg), polymyxin B(300 units) as per CLSI guidelines. [16] Colistin was tested as per CLSI guidelines. [16] *Escherichia coli ATCC 25922 & Pseudomonas aeruginosa 27853* were used as control strains.

Statistical Method:

The results were analysed using simple observation method.

RESULT

In this study, out of consecutive 300 SSI cases, 89 (29.6%) isolates met criteria of NFGNB. 252 (84%) infections were following gastrointestinal surgeries, whereas 48(16%) infections were from other surgical procedures (p-value< 0.01).

Out of the total 89 NFGNB isolated, *Pseudomonas aeruginosa* (52.8%) & *Acinetobacter baumannii* (39.3%) were the most common, followed by *Stenotrophomonas maltophilia (*3.37%), *Acinetobacter lwoffii (*2.24%) *Pseudomonas fluorescence (*1.12%), *Sphingomonas paucimobilis* (1.12%) (Table - I).

Table-I

ORGANISM	No. of isolates (Total=89)
Pseudomonas aeruginosa	47(52.8%)
Acinetobacter baumannii	35 (39.3%)
Stenotrophomonas maltophilia	3 (3.4%)
Acinetobacter lwoffii	2 (2.2%)
Pseudomonas fluorescence	1 (1.1%)
Sphingomonas paucimobilis	1 (1.1%)

Resistance pattern (Table-II and Table-III) reveals, 93% *Acinetobacter baumannii* were resistant to ampicillin, 97.10% to cefepime, *76*% to meropenem, 28% to cefoperazone-sulbactam combination, whereas significantly colistin resistance was seen in 2.85%.

Among *Pseudomonas aeruginosa* isolates, 91% were resistant to ampicillin, 78% to levofloxacin, while only 30.69% to cefoperazone-sulbactam combination and 5% isolates to meropenem. All *Pseudomonas aeruginosa* isolates were sensitive to polymyxin B and colistin.

All the 3 isolates of *Stenotrophomonas maltophilia* were resistant to both levofloxacin and cotrimoxazole.

Different types of pigments produced by the NFGNB help in their identification upto species level. Ninety-four percent (94%) of *Pseudomonas aeruginosa* isolates have produced pyocyanin pigment and rest 6% produced fluorescein.

All *Acinetobacter baumannii* isolates could survive and multiply at 10ºC, a collateral finding.

Table no. II

ANTIBIOTICS	PERCENTAGE RESISTANT (n=35)
Ampicillin	93% (n=33)
Amikacin	45% (n=16)
Cephotaxime	81% (n=28)
Cefepime	97.10% (n=34)
Cefoperazone-sulbactam	28% (n=10)
Cotrimoxazole	87% (n=30)
Gentamicin	69% (n=24)
Imipenem	82% (n=29)
Meropenem	76% (n=27)
Levofloxacin	71% (n=25)
Piperacillin-tazobactam	44% (n=15)
Colistin	2.85% (n=1)

Table no.II: Antibiotic resistance pattern of *Acinetobacter baumannii*

Table no. III

ANTIBIOTICS	PERCENTAGE RESISTANT (n=47)
Amikacin	32.7% (n=15)
Ampicillin	91% (n= 43)
Ceftazidime	72.3% (n= 34)
Cefoperazone-sulbactam	30.69% (n= 14)
Gentamicin	74% (n= 35)
Levofloxacin	78% (n= 36)
Imipenem	9% (n=4)
Meropenem	5%(n=2)
Piperacillin-tazobactam	51% (n=24)
Polymixin B	0% (n=0)
Colistin	0% (n=0)

Table no.III: Antibiotic resistance pattern of *Pseudomonas aeruginosa.*

DISCUSSION

NFGNB that were considered to be contaminants in the past have now emerged as important healthcare-associated pathogens [15,17] and are being increasingly associated with many HAI including SSI. The complex physicochemical properties of these organisms necessitate a battery of tests for their precise identification. [7] In addition there is still much confusion regarding the taxonomic status of many of these NFGNB and identification of these nonfermenters have often been neglected. [9,18]

We intended to identify commonly encountered, clinically significant NFGNB from SSI along with their antimicrobial susceptibility pattern.

Pseudomonas aeruginosa and Acinetobacter species are known to be common nosocomial pathogens. [17,9] Similar observations have also been made by our study. NFGNB belonging to *Pseudomonas* species: *P. aeruginosa* and *P. fluorescens*, along with the *Acinetobacter* species accounted for 95% of the isolates during this six months period i.e. from January 2018 to June 2018.

Different types of pigments are produced by NFGNB, which are helpful in making a species identification. *Pseudomonas aeruginosa* produces pyocyanin pigment in 92-97% of strains. In our study 94% of *P. aeruginosa* isolates have produced pyocyanin pigment.

Pseudomonas aeruginosa isolates in this study were fairly susceptible (average 91%) to polymyxin-B, colistin, imipenem, meropenem, and cefoperazone-sulbactam combination in total. This is in contrast to a study from Chandigarh [19] which shows NFGNB exhibited 71.4% and 75% resistance to amikacin and 38.3% and 62.5% to netilmicin in tracheal and bronchial specimens, respectively. and another study from Bangalore [20] which shows 36.4% of NFGNB were resistant to imipenem. 42% percent of *P aeruginosa* and 18.5% of *A baumannii* were imipenem resistant.

Present study showed *Acinetobacter baumannii* isolates were fairly high susceptible to colistin (97.15%), cefoperazone-sulbactam (72%) followed by piperacillin-tazobactam (56%) (Table-II) but showed great degree of resistance to antimicrobials commonly used to treat Gram negative flora like amikacin(45%), gentamicin (69%), levofloxacin(71%), imipenem (82%), meropenem (76%). Even one of the *Acinetobacter baumannii* isolate was resistant to colistin, the last therapeutic resort for high antimicrobial resistant infections, an alarming phenomenon.

CONCLUSION

Bottom lines of the study are high frequency isolation (29.6%) of non fermenting organisms from SSI, relatively better situation as regards antimicrobial susceptibility from Class I cities of India. But the most worrying phenomenon is emergence of colistin resistant Acinetobacter in one isolate which is being reported by contemporary other studies from India. A collateral information of ability to grow at 10^0C, consistently by all 35 *Acinetobacter baumannii* isolates could be included in the diagnostic tests for species identification of Acinetobacter.

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