Original article:

Nonfermentative gram negative bacilli- characterisation and antibiotic resistant pattern study from a tertiary care hospital

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ABSTRACT

Introduction: Non-fermentative gram negative bacilli (NFGNB), frequently considered as contaminants, have emerged as important nosocomial pathogens causing opportunistic infections. The rise in incidence of these infections and resistance to a wide range of commonly used antibiotics necessitates their characterization up to species level. Also, multidrug resistance exhibited by NFGNBs poses a major clinical problem. The objective was to study the species characterization and drug resistance patterns of non-fermenters in various clinical specimens and to estimate the incidence of multidrug resistance of NFGNBs.

Materials & methods: Various clinical specimens like pus, urine, blood, broncho-alveolar lavage, endotracheal aspirations, drain tip and cerebrospinal fluids collected from hospital patients were studied. The non-fermenters were identified using a standard protocol that included tests for motility, oxidase production, oxidation-fermentation test for various sugars, and gelatin liquefaction. Antimicrobial sensitivity was determined using Kirby Bauer disc diffusion method. Multidrug resistant (MDR) isolates of the non-fermenters were estimated. Descriptive statistics were used using Microsoft Excel Spreadsheet.

Results & conclusion: Incidence of multidrug resistant nonfermenters was 75.6%. 7.7% of nonfermenters were pan-drug resistant showing resistance to all commonly used antibiotics. Combination therapy of Piperacillin-Tazobactum, Quinolones-Aminoglycosides and Imipenam–Aminoglycosides were recommended for treatment of infections by nonfermenters. Potentially toxic agents like Colistin and Polymixin B were the only options available for treating the multi-resistant isolates.

Keywords: non-fermenters, clinical samples, antibiotic resistance, multidrug resistance

INTRODUCTION

Non-fermentative gram negative bacilli (NFGNB) are a ubiquitous group of aerobic, non-spore forming organisms. Although frequently considered as contaminants, most of them have emerged as important nosocomial pathogens causing opportunistic infections in immmuno-compromised hosts. Longer duration of hospitalization and prolonged antibiotic therapy are the predisposing factors for infection with non-fermenters. Humidifiers, ventilator machines, dialysate fluids and catheter devices in the hospital environment have provided opportunities for these organisms to establish infection [1]. The infections caused by them include urinary tract infections, wound infections, septicemia, pneumonia, osteomyelitis, meningitis etc. Chronic infection, longer duration of hospitalization and prolonged antibiotic therapy has increased the incidence of these infections necessitating their characterization up to species level. Multidrug

resistance exhibited by them pose a major clinical identification and institution of appropriate treatment is necessary to reduce the morbidity and mortality due to these organisms in hospitalized patients [2,3,4, 5].

MATERIALS & METHODS:

This cross sectional study included 156 clinical specimens like pus, urine, blood, broncho-alveolar lavage, endotracheal aspiration, drain tip and cerebrospinal fluid collected from patients of a tertiary care hospital. Antimicrobial sensitivity was determined using Kirby Bauer disc diffusion method. Multidrug resistant (MDR) isolates of the non-fermenters were estimated. MDR isolate was defined as resistant to three or more drugs of therapeutic relevance.

RESULTS

A total of 156 strains of NFGNB from various clinical samples were studied for their role in hospital infections. Higher incidence of non-fermentative isolates was observed in the age group of 21-40 years (51.3%) with a preponderance of males (70.5%). Pus (40.4%) and urine (30.8%) among the various clinical samples showed the highest incidence of non-fermenters and the predominant source of these infections were from surgical wards (25.5%). Infections by NFGNB were frequently associated with risk factors and Intensive Care Units formed a significant source of infections by these organisms (16.9%).

Pseudomonas aeruginosa was the predominant isolate (58.9%) among the non-fermenting gram negative bacilli, followed by Acenetobacter baumanii (29.5%), Stenotrophomonas maltophilia (4.5%), Pseudomonas fluorescens (2.6%), Alcaligenes fecalis (1.9%), Burkholderia cepacia (1.3%) and Weeksella virosa (1.3%). Ps. aeruginosa (44.6%) and Ps. fluorescens (100%) were predominantly isolated from pus; A.baumanii (41.3%), W.virosa (100%) and A.fecalis (100%) from urine; S. maltophilia (57.2%) from tracheal aspirates and B. cepacia (50%) from pus and sputum. The distribution of nonfermeners in various clinical specimens is shown in Table 1.

Non-fermenters were most sensitive to Imipenam and Meropenam (87.8%) , followed by Piperacillin-Tazobactum(83%), Ceftazidime(59.6%), Amikacin (62.8%) and Quinolones (53.2%). Resistance was observed for Cefotaxime (80.8%) and Gentamicin (72.5%). Incidence of multi-drug resistant non-fermenters was 75.6%. 7.7% of nonfermenters were pan-drug resistant showing resistance to all the commonly used antibiotics. Antibiotic sensitivity pattern of non-fermenters is shown in Table 2.

DISCUSSION:

In pus samples, Ps. Aeruginosa was the most frequently isolated (65%), followed by A.baumanii (22%), Ps. fluorescens (6.3%), S.maltophilia (5%), and B.cepacia (1.5%). Vijaya D et al reported 78.9% incidence of *Ps.aeruginosa* and 6.1% incidence of A*cinetobacter sp*. in a study of 133 NFGNB in clinical specimens. Ashutosh S et al reported an incidence of 63% and 9% in a similar study in UP [6,7].

Among urine samples, 50% had *Ps. aeruginosa* and 40% had *A.baumanii*. This is in concordance with other Indian authors - Meharwal et al, Taneja et al and Patel PH et al. [8,9, 10] . *A.fecalis* (3/3) and *W.virosa* (2/2) were exclusively isolated from urine samples only. In respiratory specimens (sputum, tracheal aspirates and BAL), *Ps.aeroginosa* and *A.baumanii* were most frequently isolated. The predisposing factors in the respiratory tract observed in the present study were COPD, Chronic bronchitis

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and underlying chronic diseases. Studies by Hseuh PR et al, Italy, Mastoraki A et al, Veenu et al, Ferrara et al, Arora et al, have reported *Ps. aeruginosa* as the most frequent isolate from respiratory tract across the world, however, with a varying incidence rates [11,12,13,14,15]. 8 of 10 blood specimens grew *Ps.aeroginosa* and the other 2 *A.baumanii*. One CSF sample and one drain fluid sample showed *Ps.aeroginosa.*

A. baumanii, a major species of *Acinetobacter,* was the second most frequent NFGNB isolated from 46 of 156 samples (30%). Acinetobacter baumannii has emerged as a highly troublesome pathogen for many institutions globally. As a consequence of its immense ability to acquire or upregulate antibiotic drug resistance determinants, it has justifiably been propelled to the forefront of scientific attention [16].

S.maltophilia is an emerging nococomial pathogen in hospital settings. The significance of this organism lies in its intrinsic multidrug resistance [17]. Seven out of 156 (4.5%) nonfermenting gram negative bacilli were S.maltophilia in this study. This is less compared to the reports in China (9.2%) [18,19] but comparable to other Indian reports - 3.35% (Vijaya et al [6]) and 1% (Meharwal, Taneja, and Veenu et al [14,20,21]. Four isolates of *S.maltophilia* were from endotracheal aspirates in this study. A study by Wu CL et al., [19] had reported *S.maltophilia* as the third most common organism isolated from endotracheal aspirates . Kollef et al [22] reported that isolation of this high risk pathogen is an important predictor of mortality in Ventilator Associated Pneumonia. In the present study, the other 3 isolates were obtained from pus (2 from diabetic foot ulcer & 1 from postoperative wound swab) similar to the studies by Veenu et al. *S.maltophilia* has been frequently isolated from wounds and other skin lesions. [14] *P.fluorescens* is also a common isolate in various clinical specimens mentioned in the literature . In the present study, 4/156 NFGNBs (2.6%) were P.fluorescens. All of them were isolated from pus. A study by Sheretz et al., reported 2.38% incidence in pus. [21]

B.cepacia has emerged as a significant respiratory pathogen and especially from sputum of cystic fibrosis patients. Study done in Chandigarh [11] showed that majority of these isolates were from blood. In the present study, one was isolated from sputum and the other from diabetic foot ulcer. *A. fecalis* is a pathogen in hospital acquired infections especially in persons with underlying diseases. In the present study 3/156 strains were isolated from urinary tract infections. Vijaya et al [6] and Veenu [14] et al isolated 3 and 1 isolates from urine respectively. In the present study 2 *W. virosa* isolates were obtained from urine. Meharwal et al [20] isolated one strain of *W.virosa* from urine.

Multidrug resistance is a major problem with non fermenting gram negative bacilli and so the infections caused by them are very difficult to be treated. In the present study, 75.6% of NFGNB were resistant to 2 or more drugs of relevance and *Ps aeruginosa* (77%), *A. baumanii* (78%) & *S.maltophilia* (100%) were the most common multidrug resistant strains. This is higher than the studies in India reported previously in the year 1998 (Vijaya et al) and 2000 (Veenu et al) at 31% & 62% respectively but comparable to the study by Patwardhan et el in 2008.[6,14,24]

While *S.maltophilia* is intrinsically resistant to antibiotics, *Ps aeruginosa & A.baumanii* acquire resistance by many different mechanism like Extended spectrum of betalactamases (ESBL) and Metallobetalactamases(MBL). This is of concern as

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efficacious antimicrobial options are limited. *Acinetobacter* was found more multidrug resistant than *Pseudomonas* in our study and this finding correlated with the studies by Tanya et al (53% and 49%) and Homer et al (62% and 58%). [25]

Multidrug resistant organisms were susceptible to Piperacillin – tazobactum (62.3%), and Carbapenams (82.7%). Maximum resistance was seen with Gentamicin (94.9%) and Cefotaxime (95.9%). The present study observed highest resistance of NFGNB against Cefotaxime & Gentamicin antibiotics which are commonly used drugs . This necessitates the judicious use of these antibiotics in empirical therapy. Maximum sensitivity was observed with newer agents like Carbapenams and Pipercillin-Tazobactum. Moderate sensitivity was seen with Aminoglycosides and Fluroquinolones. Major risk of using monotherapy is the emergence of antibiotic resistant bacteria as observed in the present study which showed high rate of multidrug resistance and ESBL producers. Carbapenamase resistance, though not high was still observed as an emerging drug resistant mechanisms in the NFGNB from this hospital.

Antibiotic therapy either empiric or documented is based upon antibiotic combination supplemented by knowledge of local epidemiology of resistance and susceptibilities in choosing a suitable combination.[26,27,28,29,30]Therefore combination antibiotic therapy like Piperacillin-Tazobactum, Quinolones-Amikacin, Imipenam-Amikacin would be an ideal choice of therapy on the basis of antibiotic susceptibility testing as observed in this study along with an adequate infection control measures especially in the surgical and ICU units [31,32,33]

CONCLUSION

Observations from the present study showed the aerobic NFGNB which are usually considered as contaminants are now emerging as important nosocomial pathogens. The various clinical specimens from whom they were isolated proved their existence in all sites leading to a range of diseases. Different sensitivity pattern and multidrug resistance exhibited by non-fermenters pose a great problem in treating these infections. ESBL and MBL production by these organisms lead to high morbidity and mortality as we are left with the only option of treating them by potentially toxic agents like Colistin and Polymyxin B. Awareness of their entry into a hospital environment is the first step that Clinical Microbiologists can take to address this problem. Care in detection, evaluation of effective antibiotic options, and judicious use of antibiotics by instituting antibiotic policy of combination therapy and rigorous infection control measures will help to fight against these multidrug resistant non-fermenters in the effective management of patients.

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