DOI: https://doi.org/10.53350/pjmhs202317285

ORIGINAL ARTICLE

The Threat of Carbapenem-Resistant Non-Fermenting Gram Negative Bacteria (NFGNB)

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ABSTRACT

Aim: To Isolate Non fermenting Gram negative bacteria (NFGNB) from clinical specimens of infected patients in a tertiary care hospital and then determine the antimicrobial susceptibility of these isolates to Carbapenems
Methodology: It was a Retrospective Cross-Sectional study conducted in the department of Pathology, of a tertiary care hospital, Lahore from December 2020-November 2021. The study included 4320 specimens received in Microbiology lab, for culture and sensitivity and consisted of urine, sputum, bronchial washings, pus, wound swabs, ETT, Folly's catheter tip, HVS, body fluids, blood etc. All other specimens were inoculated on Blood agar (Oxoid UK), Mac Conkey agar (Oxoid UK), and urine was inoculated on CLED (Oxoid UK). The culture plates were incubated overnight at 37°C. Bacterial identification was achieved by gram staining, and biochemical profile. Analytical profile index API-20NE (Biomerieux, France) was used for non fermenting gram negative bacteria (NFGNB).
Results: Four thousand three hundred and twenty specimens were processed and Gram negative bacteria were isolated in 651 cases constituting 15.1%. Out of these GNR, 130(19.97%) NFGNB were isolated. Maximum number of NFGNB were obtained from patients admitted in Surgery ward constituting 46 cases followed by Medical ward consisting of 38 cases, 15 cases each from ICU and OPD. Pus/wound swabs yielded majority of NFGNB 58(44.6%), followed by urine 33(25.3%), sputum 15(11.5%), and HVS 14(10.7%). *Pseudomonas aeruginosa* exhibited 35.29% and 32.35% resistance to meropenem (MEM) and imepenem (IPM) respectively.
Conclusion: NFGNB are emerging pathogens responsible for variety of infections. These resilient pathogens have developed alarming resistance against the previously considered last therapeutic resort, carbapenems.
Keywords: NFGNB, Carbapenems, Kirby Bauer disc diffusion method, CLSI, meropenem, imepenem,

INTRODUCTION

Antimicrobial drug resistance (AMR) has been declared by the World Health Organization (WHO) as one of the three worst threats to human life globally[1]. Among antibiotic resistant bacteria, gram negative bacilli particularly non-fermenting Gram-negative bacteria (NFGNB) are placed at the top of this epidemic. Centers for Disease Control and Prevention (CDC) has reported *Acinetobacter baumannii* and *Pseudomonas aeruginos* as 'urgent' and 'serious' threats of human concern respectively[2].

A major concern regarding these pathogens is their intrinsic resistance to a number of antimicrobials. This characteristic leaves us with a very limited arsenal of therapeutic options. Among the best available antibiotics for resilient bacteria are Carbapenems. Unfortunately, emerging resistance to carbapenems among Gram negative bacteria has been encountered in the recent past and is now turning into a global issue[3]. Carbapenem-resistant *Acinetobacter baumannii*, carbapenem-resistant *Pseudomonas aeruginosa*, and carbapenem resistant Enterobacteriaceae (CRE) have been ranked as highest category in the Global priority list of pathogens by WHO (The World Health Organization)[4].

This worldwide epidemic requires prompt identification and surveillance of the pathogens causing significant number of untreatable infections[5]. Among hospitalized patients, particularly seriously ill patients in ICUs, carbapenems like imepenem and meropenem are considered the final therapeutic resort. But, the rising carbapenem-resistance poses a major challenge in clinical practice[6]. The most significant clinical pathogens among NFGNB are *Pseudomonas aeruginosa, Acinetobacter baumannii and Stenotrophomonas maltophilia*, being responsible for majority of hospital acquired infections and associated with therapeutic failures. As a result, the efficacy of carbapenems, previously considered as mainstay of treatment of life threatening infections,

Received on 24-08-2022
Accepted on 19-01-2023

is questioned and it necessitates regular surveillance, judicious use of antimicrobials, and adherence to infection prevention and control measures, to limit infections by resilient pathogens[7,8]. Infections caused by resistant pathogens results in increased death rate as these are linked to efforts of treating the patients with ineffective drugs[9]. Introducing the β-lactam–β-lactamase inhibitor combination strategies such as Ceftazidime /avibactam and ceftolozane/tazobactam gave a ray of hope against this alarming issue but resistance against these newer drugs have already been encountered[10,11].

Since there is a lack of promising drug development in the near future, it is the need of hour to limit the use of antibiotics in accordance with susceptibility reports only. The practice might help to conserve this group of antibiotics for patients ill with serious, difficult to treat infections. This study was designed to identify the susceptibility of NFGNB to carbapenems in a tertiary care hospital. This study might prove useful to design empiric therapy for hospitalized patients where carbapenems are given as first choice.

The objectives of the study were to isolate non fermenting Gram negative bacteria (NFGNB) from clinical specimens of infected patients in a tertiary care hospital and to determine the susceptibility of Non fermenting Gram negative bacteria (NFGNB) to carbapenems

METHODOLOGY

It was a retrospective cross-sectional study carried out in the Pathology Department of a tertiary care hospital, Lahore over a period of 1 year from December 2020- November 2021. It included 4320 specimens received in Microbiology lab for culture and sensitivity from outpatient department, admitted ward patients and patients in ICUs, showing signs and symptoms of infections. The

specimens were urine, sputum, bronchial washings, body fluids, pus/ wound swabs, ETT, Folly's catheter tip, HVS, blood etc. Institutional ethical and research committee approved the project for research purpose.

All other specimens were inoculated on Blood agar (Oxoid UK) and Mac Conkey agar (Oxoid UK). CLED (Oxoid UK) was used for urine inoculation. The culture plates were incubated for 24 hours at 37°C. The bacterial isolates were identified by gram staining and biochemical profile. Analytical profile index API-20NE (Biomerieux, France) was used to identify Non fermenting gram negative bacteria (NFGNB) according to manufacturer's protocol. The antimicrobial sensitivity of the isolates using antibiotic discs Imipenem, IPM (10µg) and Meropenem, MEM (10µg) was assessed by Kirby Bauer disc diffusion method using CLSI guidelines[12].

Data analysis: The data was entered and analyzed by SPSS version 24. Frequencies and percentages were evaluated for the study variables. The p-value of ≤0.5 was taken as statistically significant.

RESULTS

A total of 4320 specimens were received in Microbiology laboratory for culture and sensitivity over a period of 1 year (December 2020 to November 2021) from outdoor, ICUs and various wards. Gram negative bacteria were isolated in 651 cases constituting 15.1%. Out of these GNR, 130 (19.97 %) NFGNB were isolated.

Figure 1 shows the distribution of isolated NFGNB in both genders. It is evident that 48% of males and 52% of females yielded NFGNB, hence there is no gender predisposition.

Figure 2 demonstrates the distribution of NFGNB in various wards, OPD and ICU. This figure reveals that maximum NFGNB were isolated from patients admitted in Surgery ward constituting 46 cases followed by Medical ward consisting of 38 cases. Fifteen cases each from ICU and OPD, 13 cases from Gynaecology Department and 3 cases from Orthopaedics Department. exhibited *Pseudomonas aeruginosa* and *Acinetobacter sp.*

Figure 3 gives information regarding various clinical specimens from which NFGNB (*Acinetobacter species and Pseudomonas aeruginosa*) were isolated. This figure exhibits that maximum positive growth was isolated from pus/wound swabs, 58(44.6%), followed by urine 33(25.3%) , sputum 15(11.5%) and HVS 14(10.7%).Three cases each from Folly's catheter tip and tissue ,while 2 cases each from bronchial washings and ETT revealed NFGNB.

The susceptibility of isolated NFGNB is shown in Table 1. *Pseudomonas aeruginosa* exhibited 35.29% and 32.35% resistance to meropenem (MEM) and imepenem (IPM) respectively. However, very alarming sensitivity results were obtained for *Acinetobacter sp* as 85.7% an 82.1% resistance was observed for meropenem and imepenem respectively.

Figure 1: Gender distribution of NFGNB

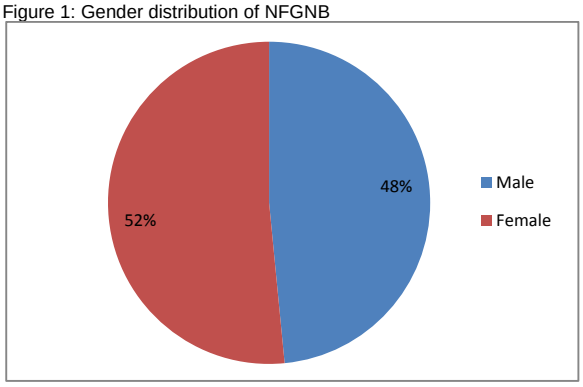

Figure 2: Distribution of NFGNB in various wards ,OPD and ICU

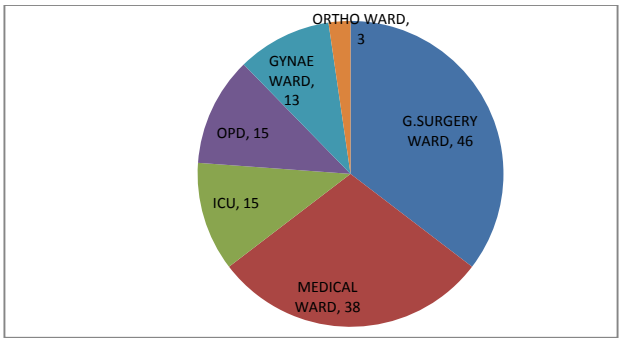

Figure 3: Distribution of different clinical specimens yielding NFGNB

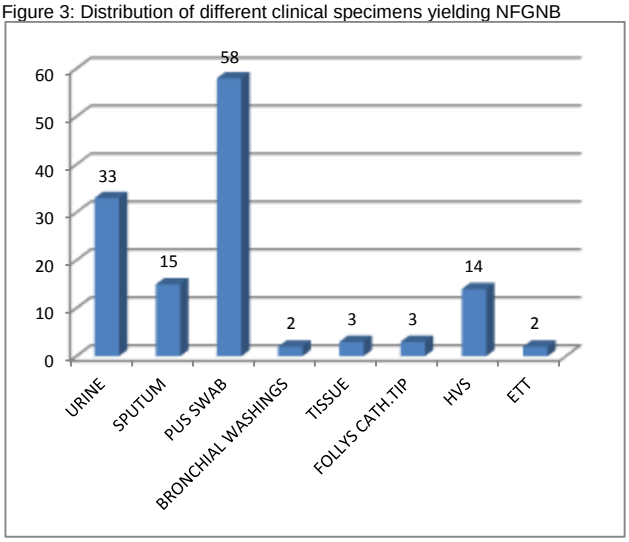

Table 1: Susceptibilty of NFGNB to carbapenems

	***Pseudomonas aeruginosa* n= 102**		***Acinetobacter species* n= 28**	
	Resistant R	**Sensitive S**	**Resistant R**	**Sensitive S**
MEM	36 (35.29%)	66 (64.70%)	24(85.71%)	4(14.28%)
IPM	33 (32.35%)	69 (67.64%)	23(82.14%)	5(17.85%)

DISCUSSION

Since bacteria causing infections and their antibiotic resistance pattern varies from place to place and changes over the course of time, it is imperative to investigate the pathogens and their sensitivity profile to optimize the treatment of infections. Resistance to carbapenems particularly by the emerging pathogens NFGNB, is a major obstacle, hampering the management of infections particularly in hospitalized patients. Hence, the current study aimed at isolating NFGNB in a tertiary care hospital and then determine their sensitivity for carbapenems. Among NFGNB, *Pseudomonas aeruginosa, Acinetobacter baumannii, and Stenotrophomonas maltophilia* are responsible for most of the infec *Acinetobacter baumannii* (44%), *Pseudomonas aeruginosa* (40.1%), and *Burkholderia cepacia* (8.2%)[13]. The findings correspond to our study results as our isolated NFGNB exclusively consists of *Pseudomonas aeruginosa* 102 (77.86%) and *Acinetobacter sp* 28 (21.37%).

The current study shows that both the genders are almost equally affected by NFGNB as 48% males and 52% females were found to be infected by *Pseudomonas aeruginosa* and *Acinetobacter* species. This is unlike a study assessing Clinico-epidemiological profile of *Acinetobacter sp* and *Pseudomonas* infections. The study observed that around 61% males and 39% females were infected with NFGNB respectively[14].

As far as ward-wise distribution is seen, maximum NFGNB were isolated from patients admitted in Surgery ward constituting 46 (35.3%) cases followed by Medical ward consisting of 38(29.23%) cases. Fifteen cases (11.5%) each from ICU and OPD, 10% from Gynaecology ward and only 2.3% from Orthopaedics ward exhibited *Pseudomonas aeruginosa* and *Acinetobacter sp. Yadav et al,2020* reported that 39.1% NFGNB were obtained from ICU patients, 21.9% each from surgical and medical wards and the remaining from orthopedics, pediatrics and burn wards[13]Another study conducted in 2018 documented percentage yield of *Acinetobacter sp* from various wards, reporting 30.5% from Surgery ward, 19% from Medicine ward, 21.9% from ICU and 15.2% from Gynaecology ward[15].

Our study results exhibit that majority of *Acinetobacter sp* and *Pseudomonas aeruginosa* were isolated from pus swabs 58(44.6%), followed by urine 33(25.3%), and sputum 15(11.5%). The findings of present study agree with a study in Nepal as the three specimens yielding majority of NFGNB were specimens collected from lower respiratory tract infections , pus/swab, and from urinary tract[13]. Mekonnen et al 2021 reported 38.9% pus swabs, 31.5% urine and 29.5% blood samples yielding *Pseudomonas aeruginosa* and *Acinetobacter sp[16].* Another study conducted on *Acinetobacter sp,* also concluded that pus swabs revealed maximum number of *Acinetobacter isolates,* followed by sputum, and urine[15].

The susceptibility results of *Pseudomonas aeruginosa* reveal that 35.29% and 32.35% isolates were resistant to meropenem and imepenem respectively. *The results are in accordance with* a case control study by Fatima et al which isolated *P. aeruginosa* exhibiting 27.8 to 41% resistance to carbapenems.[17] However, these results are in contrast to *findings obtained by* E.K. Oladipo 2018, reporting a very low percentage of only 2.1% resistance to imepenem in *Pseudomonas aeruginosa*[18].

Our study revealed even higher resistance of *Acinetobacter i*solates for carbapenems. Meropenem was found resistant against 85.71% and imepenem against 82.14% isolates of *Acinetobacter sp.* Similar results were reported in 2019 by *Maria et al,*showing 83.3% carbapenem resistance among *Acinetobacter sp*[19]. Other studies also reveal poor susceptibility results for carbapenems against NFGNB, documenting around 18-20% sensitivity of *Acinetobacter sp* and 38-42% sensitivity of *Pseudomonas aeruginosa* to carbapenems.[13] Even poorer results were reported in other recent studies on respiratory tract pathogens documenting around 78% -79.4% carbapenem resistance among NFGNB[20,21].

The situation is quite upsetting as this renders a very important drug group ineffective against these resistant, difficult to treat pathogens.

CONCLUSION

NFGNB are emerging pathogens responsible for variety of infections such as wound infection, respiratory tract infections, UTI, genitourinary tract infections etc. These resilient pathogens have developed alarming resistance against the previously considered last therapeutic resort, carbapenems.

Future prospects: The study is an eye opener; calls for judicious use of antibiotics and strict adherence to infection prevention and control measures. It might also help clinicians design empiric therapy for hospitalized patients where carbapenems are given as first choice, and highlights the role of Culture and sensitivity.

Limitations and recommendations: Since it is a single –centre study, generalization of results need more large scale studies.

Authors' contribution: SI: Conception, planning of research, Data Collection, writing of manuscript, and Discussion, **SI**: Help in references, Discussion, **SS, SZH:** Proof Reading and critically revised the paper in keeping with important intellectual content, **IYM**: Help in references and Proof Reading, **SM**: Data Collection

Conflict of interest: Authors declared no conflict of interest.

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