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ORIGINAL ARTICLE

Prevalence and Antimicrobial Susceptibility Patterns of Non Fermenting Gram Negative Bacilli in Ismailia, Egypt

Yara E. Marei, Mohammad A. Al-Sweify, Mahmoud K. Mansour, Rania M. Kishk*
Microbiology and Immunology Department, Faculty of Medicine, Suez Canal University

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Corresponding Author:
Rania Mohammed Kishk
Assistant Prof of Medical Microbiology & Immunology, Faculty of Medicine, Suez Canal University
Tel.: **01025099921**
rankishk@yahoo.com

ABSTRACT

Background: *Non-fermenting Gram-negative rods (NFGNRs) are widely distributed in nature.* ***Objective:*** *The study was done to determine the prevalence of NFGNRs in Suez Canal University hospitals (SCUH).* ***Methodology:*** *During a one year period, batteries of 370 specimens were processed and subjected to biochemical reactions.* ***Results:*** *Among the total 305 pathogenic specimens, only 46 specimens (15%) were identified as NFGNRs. Pseudomonas aeruginosa was the commonest isolate, accounting for 45.6% of NFGNRs, followed by Acinetobacter baumannii 17.4 %. Antimicrobial susceptibility tests of NFGNRs isolates were performed and it was found that the majority were multi-drug resistant (MDR).* ***Conclusion:*** *Therefore, NFGNRs represent a considerable health problem in SCUH.*

INTRODUCTION

Non-fermenting Gram-negative rods (NFGNRs) are a group of bacilli that does not ferment glucose to obtain their energy. They either utilize glucose by oxidation or do not utilize it at all [1]. NFGNRs are widely distributed in nature [2]. The most prevalent human pathogens are *P. aeruginosa*, *A. baumannii / calcoaceticus* and *Stenotrophomonas maltophilia* [3]. NFGNRs cause variety of community-acquired as well as healthcare-associated infections including bacteremia, urinary tract infections, meningitis and ventilator associated pneumonia especially in immunocompromised or already debilitated host. The nosocomial species tend to be MDR [4]. NFGNR are innately resistant to many antibiotics and this is attributed to a variety of resistance mechanisms as production of extended spectrum ß-lactamases, enzymatic modification, over-expression of efflux pumps, porin loss and antibiotic target alterations. MDR in NFGNRs makes the treatment of infections caused by these pathogens both difficult and expensive[5].

METHODOLOGY

This cross-sectional descriptive study was carried out during a one year period from January 2013 to January 2014. Clinical isolates of NFGNRs were collected from 370 patients admitted to different wards including ICU, Neonatal ICU, Urology, Surgery, Orthopedics, Burn, and Internal Medicine and Pediatric wards in SCUH in Ismailia. According to the site and the presentation of infection, samples included 152 pus swabs, 58 sputum and endotracheal tube aspirates, 138 urine, 21 blood samples and one ascitic fluid sample. Consent was taken from each patient to use their data in the current research work. The study was approved from ethics committee in our institution.

Specimen processing:

The collected species were plated on both blood and MacConkey type I agar, incubated aerobically at 35˚C ± 2 for 24-48 hrs. Blood agar plates were examined for colonial morphology, colony size, hemolytic activity, pigmentation, Gram stain appearance and oxidase reaction. MAC agar plates were examined for non-lactose fermenting colonies or no growth after 48 hrs.

Identification of bacterial species

Only the gram negative bacilli/coccobacilli showing non lactose fermenting colonies or no growth on MAC were inoculated on Hugh and Liefson oxidation-fermentation (OF) media. Strains that produced no change in the tube overlaid with mineral oil were considered nonfermenters. *P. aeruginosa* strains were identified up to the species level by culture on Pseudomonas Pyocyanin Agar. The appearance of a green, dark green or blue-green color is strong evidence that the strain is *P. aeruginosa.* The remaining non fermentative organisms (other than *P. aeruginosa*) were diagnosed by API 20NE (Biomérieux, France).

Antimicrobial susceptibility test:

Susceptibility testing of all NFGNRs isolates was performed according to the standard Kirby-Bauer disk diffusion method on Mueller Hinton agar and interpreted as per the Clinical and Laboratory Standards Institute guidelines. The following disks were used: Ceftazidime (30µg), cefepime (30µg), meropenem (10µg), imipenem (10µg), gentamycin (10µg), tobramycin (10µg), amikacin (30µg), ciprofloxacin (5µg), levofloxacin (5µg), pipracillin-tazobactam (100µg/10 µg), azetronam (30µg), colistin (10µg),

doxycycline (30µg) and trimethoprim-sulfamethoxazole (1.25/23.75µg).

RESULTS

Out of 370 clinical specimens, 305 specimens (82.4%) showed presence of pathogenic bacterial or candidal growth and among those, 46 specimens (15%) contained NFGNRS as the pathogenic agent, while in 259 specimens (85 %), other organisms were identified. *P. aeruginosa* was the commonest isolate, accounting for 45.6% of total isolates of NFGNRs; followed by *A. baumannii* 17.4%; *P. fluorescens* 13%; *Stenotrophomonas maltophilia* 10.9 %, then *B. cepacia* 8.7 %. Only one isolate of *Achromobacter* and *P. stutzeri* were found and accounted for 2.2 % each **(figure 1)**.

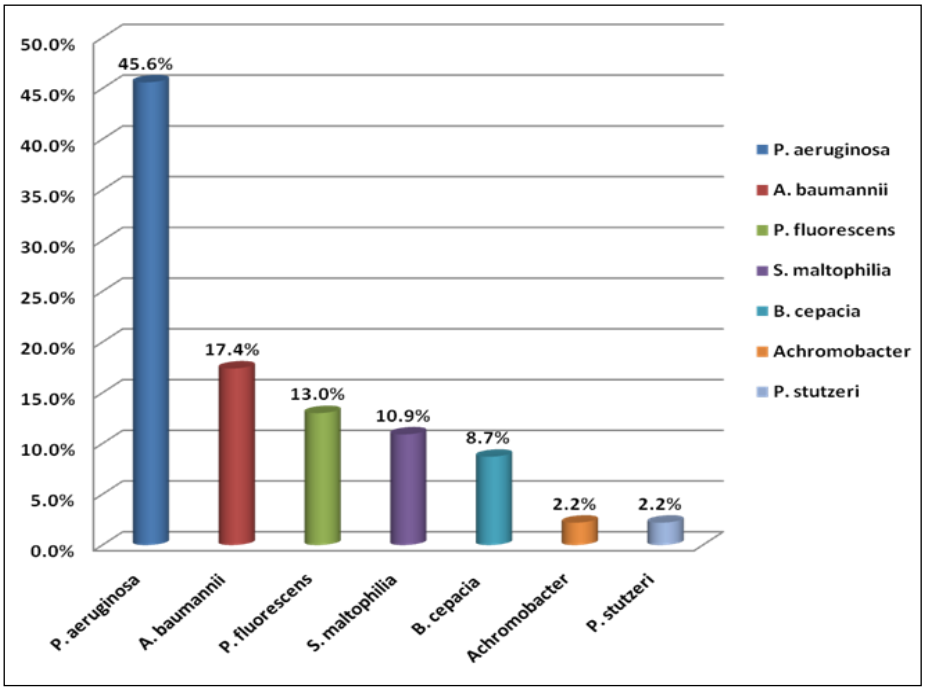

Fig. 1: Frequency of NFGN species in the collected specimens

Most of the patients infected by NFGNRs had underlying conditions as diabetes mellitus, burn, trauma, surgery, or instrumentations. The highest frequency of NFGNR (32.6%) was found in surgery wards; while the lowest frequency (6.5 %) was found in urology wards **(figure 2)**. Most of species (56.5%) were isolated from pus samples; while only (8.7%) were isolated from urine samples **(table 1)**.

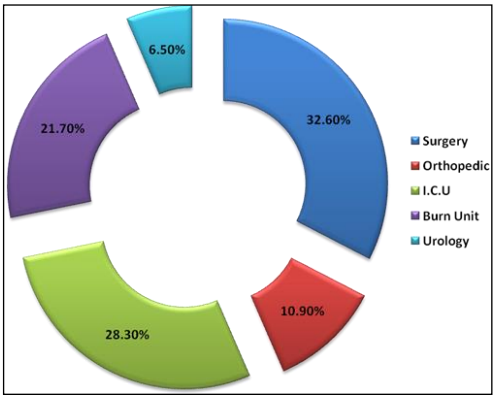

Fig. 2: Distribution of the patients infected by NFGNRs according to different wards admission.

Table 1: Distribution of NFGNRs among samples (n=46)

Organisms	Pus		Sputum		Blood		Urine	
	N$^{O.}$	% NFGNR	N$^{O.}$	% NFGNR	N$^{O.}$	% NFGNR	N^{O}	% NFGNR
***P. aeruginosa* (n=21)**	14	30.5%	3	6.5%	2	4.3%	2	4.3%
***A. baumannii* (n=8)**	3	6.5%	3	6.5%	1	2.2%	1	2.2%
***S. maltophilia* (n=5)**	2	4.3%	1	2.2%	2	4.3%	0	0%
***B. cepacia* (n=4)**	2	4.3%	1	2.2%	1	2.2%	0	0%
***Achromobacter* (n=1)**	1	2.2%	0	0%	0	0%	0	0%
***P. fluorescens* (n=6)**	3	6.5%	2	4.3%	0	0%	1	2.2%
***P. stutzeri* (n=1)**	1	2.2%	0	0%	0	0%	0	0%
Total NFGNR (n=46)	26	56.5%	10	21.8%	6	13.0%	4	8.7%

The majority of *P. aeruginosa* isolates (85.7%) were resistant to cefepime, ceftazidime, pipracillin-tazobactam and azetronam. Most of *A. baumannii* isolates showed resistance to cefepime (75.0%). The majority of *S. maltophilia* isolates (60.0%) showed resistance to doxycycline and trimethoprim-sulfamethoxazole but none of them showed resistance to levofloxacin. All isolates of *B. cepacia* showed resistance to ceftazidime but none of them showed resistance to meropenem **(table 2)**.

Table (2): Antimicrobial resistance patterns of NFGNR species

Resistance to		*P.aeruginosa* (n=21)	*A.baumannii* (n=8)	*S.maltophilia* (n=5)	*B. cepacia* (n=4)
Azetronam	N$^{O.}$	18	-	-	-
	%	85.7%	-	-	-
Ceftazidime	N$^{O.}$	18	6	-	4
	%	85.7%	75.0%	-	100.0%
Cefepime	N$^{O.}$	18	6	-	-
	%	85.7%	75.0%	-	-
Pipracillin-tazobactam	N$^{O.}$	18	6	-	-
	%	85.7%	75.0%	-	-
Imipenem	N$^{O.}$	11	2	-	-
	%	52.4%	25.0%	-	-
Amikacin	N$^{O.}$	2	1	-	-
	%	9.5%	12.5%	-	-
Meropenem	N$^{O.}$	8	2	-	0
	%	38.1%	25.0%	-	0.0%
Colistin	N$^{O.}$	1	-	-	-
	%	4.8%	-	-	-
Levofloxacin	N$^{O.}$	5	1	0	-
	%	23.8%	12.5%	0.0%	-
Gentamicin	N$^{O.}$	3	3	-	-
	%	14.3%	37.5%	-	-
Ciprofloxacin	N$^{O.}$	6	5	-	-
	%	28.6%	62.5%	-	-
Trimethoprim-sulfamethoxazole	N$^{O.}$	-	2	3	2
	%	-	25.0%	60.0%	66.7%
Doxycycline	N$^{O.}$	-	1	3	1
	%	-	12.5%	60.0%	33.3%

DISCUSSION

A total of 370 clinical specimens were collected as a representative study sample, from which we isolated 305 pathogenic organisms. Of these, 46 NFGNR strains (15%) were biochemically identified to the species level. The prevalence of NFGNR was reported in a study in Argentina as 21% of 590 various clinical specimens [6]. On the other hand, another study reported a lower prevalence of 2.2%, when they isolated 326 NFGNR isolates from 14,971 patients in a private hospital in Porto Alegre, Brasil [3].

In our study it was found that *P. aeruginosa* was the most commonly isolated NFGNR species (45.6%), followed by *A. baumannii* (17.4%), *P. fluorescens* (13.0%), *S. maltophilia* (10.9%) and *B. cepacia* (8.7 %). One isolate of *P. stutzeri and Achromobacter* were found, accounting for 2.2 %, each (figure 1). An Egyptian study was conducted in ICU, Giza chest hospital, reported that *A. baumannii* represented 16.1% of total isolates in ICU [7]. National Liver Institute, Menoufia University, Egypt reported the prevalence of *A. baumannii* was 29.3 % from cases with blood stream infections in ICU [8].

Similar results were reported in Brasil [3] where *P. aeruoginosa* accounted for 65% of all NFGNRs, followed by *A. baumannii* (16.5%), and *S. maltophilia* (9.5%). Another study in Milad Hospital (Iran) isolated 257 strains of NFGNRs of which 42.4% of isolates were *P. aeruoginosa*, 34.2% were *A. baumannii*, 18.7% were *S. maltophilia* and 4.7% were *B. cepacia* [9].

In our study, it was found that the majority of patients infected by NFGNRs were suffering from one or more underlying diseases or conditions, mainly diabetes mellitus, burns, surgery, and various invasive instrumentations. A study conducted on nine French teaching hospitals showed that the majority of patients infected by NFGNRs were immunocompromised, admitted in ICU with central venous catheter [10]. Another study showed that *P. aeruginosa* and species like *Serratia marcescens* and *A. baumannii* represented 10% of bloodstream infections in immunocompromised patients admitted to haematology/oncology hospital in Roma [11].

In our study, we found that NFGNRs were most frequently isolated from pus samples representing (58.7%) followed by sputum samples (19.6%), blood samples (13%) and urine samples (8.7%) (table1). A study conducted on patients who were infected with *A. baumannii* or *P. aeruginosa* at the Spedali Civili hospital in Brescia, Italy, showed that they were recovered more frequently from the respiratory tract, followed by the skin/soft tissue (pus), urine and blood [12]. A study in Argentina showed that most of NFGNR were isolated from respiratory secretions (36%), followed by urine (26.4%) [6].

The highest incidence of NFGNR in our study was found in surgery wards, ICU and burn unit representing 32.6%, 28.3% and 21.7%, respectively (figure 2). Enoch *et al.*[14] also isolated 43% of NFGNR among ICU patients in Japan [13]. Another study conducted in Pakistan on two separate ICU reported it to be 44% and 39%, respectively.

On observing the antimicrobial resistance patterns of various isolates in our study, most of them were MDR. Regarding *P. aeruginosa*, 85.7% of isolates were resistant to cefepime, ceftazidime, azetronam and pipracillin/tazobactam; 52.4% were resistant to imipenem; 38.1 % were resistant to meropenem; 28.6% were resistant to ciprofloxacin; 23.8% were resistant to levofloxacin and 14.3% were resistant to gentamicin**.** Only 4.8% and 9.5% of *P. aeruginosa* isolates in our study were resistant to colistin and amikacin, respectively (table 2). In a study conducted in Al-Minia University Hospitals, Egypt; the resistance to cefepime, amikacin and gentamicin was found to be 29%, 8% and 59 %, respectively [15].

In our study, 75% of *A. baumannii* isolates were resistant to cefepime, ceftazidime and pipracillin/tazobactam; 62.5% were resistant to ciprofloxacin; 37.5% were resistant to gentamicin; 25% were resistant to meropenem, imipenem, and trimethoprim-sulfamethoxazole. Only 12.5% were resistant to amikacin, doxycycline and levofloxacin. A study conducted by Al-Agamy *et al.*[16] on 40 *A. baumannii* isolates collected from two hospitals in Egypt showed that the isolates were 100% resistant to aztreonam, cefepime, cefotaxime, and ceftazidime, 5% were resistant to colistin, 45% to amikacin, 70% to imipenem, and 85% to ciprofloxacin.

Our study showed that levofloxacin and meropenem were the most effective antibiotics against *S. maltophilia* and *B. cepacia*, respectively. All isolates of *B. cepacia* showed resistance to ceftazidime, while 60% of *S. maltophilia* isolates were resistant to trimethoprim-sulfamethoxazole (the traditional drug of choice for that species) and doxycycline. Rahbar *et al.*[9] reported that 33% of *S. maltophilia* isolates were resistant to trimethoprim-sulfamethoxazole[9]. Asaad *et al.* [17] reported that 100% of isolated *S. maltophilia* were sensitive to trimethoprim-sulfamethoxazole. These variations in drug susceptibility reflect different clonal diversity as well as different antibiotic policies applied, in different hospitals even within the same country.

CONCLUSION

Infection by NFGNR represents a considerable health problem in SCUH. Since these organisms have great potential to survive in hospital environment, proper identification to species level as well as detection of resistant strains is mandatory to control their spread.

Conflict of interest: no conflict of interest

REFERENCES

1. Winn W, Allen S, Janda W, Koneman E, Procop G, Woods G, Schreckenberger P. Non Fermenting Gram Negative Bacilli. In: Winn W, Allen S, Janda W, Koneman E, Procop G, Woods G, Schreckenberger P (eds.). Color Atlas and Textbook of Diagnostic Microbiology, 2006, 6th ed, p. 305-391. USA, Lippincott Williams and Wilkins Company.
2. Lipuma J, Currie B, Lum G, Vandamme P. Burkholderia ,Stenotrophomonas, Ralstonia, Cupriavidus, Pandoraea, Brevundimonas, Comamonas, Delftia, and Acidovorax. In: Murray P, Baron E, Jorgensen J, Landry M, Pfaller M (eds.). Manual of Clinical Microbiology, 2007, 9th ed, p. 749–769. Washington, DC, American Society of Microbiology.
3. Deliberali B, Myiamoto K, Neto C. Prevalence of Non Fermenting Gram Negative Bacilli among Inpatients from Porto Alegre-RS. Jornal Brasileiro de Patologia e Medicina Laboratorial, 2011, 47(5): 529-534.
4. Kiran R, Uma Maheswari P, Madhavan R, Routray A. Phenotypic Characterization of Non-Fermentative Gram Negative Bacilli from Clinical Samples. Helix, 2012, 4: 197-201.
5. Mcgrowan J, Moellering R, Fishman N. Resistance in Non Fermenting Gram-Negative Bacteria. American Journal of Infection Control, 2006, 34:29-37.
6. Merino L, Ronconi M, Marin M, Hreñuk G, Pato A. Non Fermenting Gram Negative Bacilli: Distribution in Clinical Specimens and Antimicrobial Susceptibility. Revista Latinoamericana de Microbiologia, 1999, 41(4): 279-284.
7. El-Masry E and El-Masry H. Characterization of Carbapenem-resistant Acinetobacter baumannii Isolated from Intensive Care Unit, Egypt. Egyptian Journal of Medical Microbiology, 2018, 27 (3): 85-91
8. Hamam S and Awad S. Characterization of Antimicrobial Resistance and Prevalence of OXA Genes in the Emerging Threat Acinetobacter baumannii Causing Blood Stream Infection in ICU Patients. Egyptian Journal of Medical Microbiology, 2018, 27 (4) : 141- 148
9. Rahbar M, Mehragan H, Akbari N. Prevalence of Drug Resistance in Non Fermenter Gram-Negative Bacilli. Iranian Journal of Pathology, 2010, 5(2): 90 – 96.
10. Fihman V, Le Monnier A, Corvec S, Jaureguy F, Tankovic J, Jacquier H, Carbonnelle E, Bille E, Illiaquer M, Cattoir V, Zahar J. Stenotrophomonas maltophilia-The Most Worrisome Threat among Unusual Nonfermentative Gram-Negative Bacilli from Hospitalized Patients: A prospective Multicenter Study. Journal of Infection, 2012, 64(4): 391-398.
11. Papagheorghe R. Bloodstream Infections in Immunocompromised Hosts. Romanian Archives of Microbiology and Immunology, 2012, 71(2):87-94.
12. De Francesco M, Ravizzola G, Peroni L, Bonfanti C, Manca N. Prevalence of Multidrug-Resistant Acinetobacter baumannii and Pseudomonas aeruginosa in an Italian hospital. Journal of Infection and Public Health, 2013, 6 (3): 179-185.
13. Enoch D, Simpson A, Kibbler C. Predictive Value of Isolating Pseudomonas aeruginosa from Aerobic and Anaerobic Blood Culture Bottles. Journal of Medical Microbiology, 2004, 53:1151-1154.
14. Wroblewska M, Rudnicka J, Marchel H, Luczak M. Multidrug Resistant Bacteria Isolated from Patients Hospitalized in Intensive Care Units. International Journal of Antimicrobial Agents, 2006, 27: 285-289.
15. Gad G, El-Domany R, Zaki S. Characterization of Pseudomonas aeruginosa Isolated from Clinical and Environmental Samples in Minia, Egypt: Prevalence, Antibiogram and Resistance mechanisms. Journal of Antimicrobial Chemotherapy, 2007, 60(5):1010-1017.
16. Al-Agamy M, Khalaf N, Tawfick M . Molecular Characterization of Carbapenem-Insensitive Acinetobacter baumannii in Egypt. International Journal of Infectious Diseases, 2014, 14:1201-1210.
17. Asaad A, Al-Ayed M, Qureshi M. Emergence of Unusual Nonfermenting Gram-Negative Nosocomial Pathogens in a Saudi Hospital. Japanese Journal of Infectious Diseases, 2013, 66 (6):507-511.