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OCCURRENCE OF UNUSUAL NON-FERMENTATIVE GRAM NEGATIVE BACILLI IN INTENSIVE CARE UNITS OF A UNIVERSITY HOSPITAL, EGYPT

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RUNNING TITLE: UNUSUAL NON-FERMENTATIVE GRAM NEGATIVE BACILLI IN AN EGYPTIAN HOSPITAL

ABSTRACT

Non-fermentative Gram-negative bacilli (NFGNB) other than *Pseudomonas* and *Acinetobacter* species have emerged as nosocomial pathogens. No much data is currently available concerning the occurrence of these types of bacteria in Zagazig University Hospitals (ZUHs). In this study, the occurrence as well as the antimicrobial susceptibility pattern of unusual NFGNB obtained from clinical samples collected from intensive care units (ICUs) of ZUHs was assessed. Additionally, the genetic relatedness among the most prevalent unusual NFGNB species was studied. Results: Out of 516 non-repeated clinical sample, 97 NFGNB (18.7%) were isolated. Among them, 17 unusual NFGNB were identified by API 20NE, accounting for 17.5% of NFGNB and 3.3% of all tested samples. Within the unusual NFGNB, *Burkholderiacepacia*complex (*Bcc*) was the most prevalent species accounting for 94.1% of NFGNB and 3.1% of total samples. This was followed by *Burkholderia pseudomallei* (*B. pseudomallei*) which accounted for 5.9% of NFGNB and 0.2% of all obtained specimens. Tigecycline antibiotic was the most effective antibiotic against *Bcc* isolates (68.8% susceptibility) in disc diffusion method. After random amplified polymorphic DNA (RAPD) testing, the obtained *Bcc* isolates were found to be genetically diverse. This highlights *Bcc* as an emerging nosocomial pathogen in ICUs of ZUHs. Continuous monitoring of the occurrence of *Bcc* in ICU as well as in other hospital wards is warranted.

Key words: "Unusual", "Non-fermentative", "gram-negative bacilli", "intensive care unit"

Abbreviations: *Bcc*; *Burkholderia cepacia* complex, CF; cystic fibrosis, ICUs; intensive care units, MIC; minimal inhibitory concentration, NF; non-fermenters, NFGNB; non-fermentative gram-negative bacilli, RAPD; random amplified polymorphic DNA, ZUHs; Zagazig University Hospitals

OCCURRENCE D'INHABITUEL NON – FERMENTAIRE BACILLES GRAM EN UNITES DES SOINS INTENSIFS D'UN HOPITAL UNIVERSITAIRE, EGYPTE

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TITRE COURANT : NON – FERMENTAIRE BACILLES GRAM NEGATIF DANS UN HÔPITAL EGYPTIAN

RESUME

L'espèce non – fermentaire bacilles Gram négatif (NFGNB) autre que *Pseudomonas* et *Acinetobacter*ont émergé comme agents pathogènes nosocomiaux. Il n'y a pas beaucoup de données actuellement disponibles concernant la présence de ces types de bactéries aux hôpitaux universitaire de Zagazig (ZUHs). Dans cette étude, l'occurrence ainsi que le motif de la sensibilité aux antimicrobiens des NFGNB inhabituelle obtenue d'échantillons cliniques prélevésd'unités de soins intensifs de ZHUs ont été évalués. Par ailleurs, la parenté génétique entre lesespèces les répandues de NFGNB inhabituelles a été étudiée.

Résultats : Sur 516 échantillons cliniques non – répétés, 97 NFGNB (18,7%) étaient isolés. Parmi eux, 17 NFGNB inhabituelle ont été identifié par API 20NE, représentant 17,5% de NFGNB et 3,3% de tous les échantillons testés. Au sein de NFGBN, le

complexe *Burkholderiacepacia* était l'espèce le plus répandureprésentant 94,1% de NFGNB et 3,1% des échantillonstotal. Ceci a été suivi par *Burkholderiapseudomallei (B. pseudomallei)* lequel représentait 5,9% de NFGNB et 0,2% de tous spécimens obtenus. Tigecycline antibiotique était l'antibiotique le plus efficace contre les isolats *Cci* (68,8% susceptibilité) dans la méthode de diffusion sur disque. Suite a des tests aléatoires d'ADN polymorphique amplifie (RAPD), les isolats *Cci*obtenus se sont trouvés d'êtregénétiquement divers. Ceci souligne *Cci* comme un pathogènes nosocomiaux émergents en USI de ZUHs. La surveillance continue de l'occurrence de *Cci* en USI, ainsi que dans d'autres services hospitaliers est justifiée.

Mots clés : Inhabituelle, Non fermentaire, bacille gram négative, unité des soins intensifs.
Abréviations : Cci :le complexe Burkholderiacepacia ; CF : fibrose kystique ; USI : Unités des soins intensifs ; MIC : concentration minimal inhibitrice ; NF : non fermenteurs ; NFGNB : non fermentaire bacilles gram négative ;RAPD ; ZUHs : Hôpitaux universitaire de Zagazig.

INTRODUCTION

Non-fermentative gram-negative bacilli (NFGNB) are a diverse group of aerobic non spore-forming bacilli. They usually present as saprophytes in the environment, particularly, in soil and water. Although being saprophytic in nature, NFGNB has emerged as important healthcare-associated pathogens. Their resistance to disinfectants, in addition to their potential to spread from patient to patient via fomites, or the hands of medical personnel have made them of great concern in hospital settings [1] [2].

The majority of earlier studies have only focused on *Pseudomonas* spp. and *Acinetobacter* spp. being the most frequently isolated NFGNB, considering the unusual group as having a minor clinical significance. Nevertheless, serious infections due to NFGNB other than *Pseudomonas* spp. and *Acinetobacter* spp. are currently being reported with increasing frequency forming a significant contribution to in-hospital mortality, particularly, in immunocompromised patients[3] [4].

Unfortunately, human infections caused by these bacteria are underestimated. This is partly due to their complex identification along with their frequent misidentification by phenotypic methods commonly used in clinical laboratories. Furthermore, the results obtained for some of these organisms by disc diffusion method do not correlate with successful clinical outcome nor with those obtained by the more accurate minimal inhibitory concentration (MIC) methods [5] [6].

As no much data is currently available concerning the occurrence of these types of bacteria in Zagazig University Hospitals (ZUHs), this study aimed to assess the prevalence as well as the antimicrobial susceptibility pattern of unusual NFGNB isolated from intensive care unit (ICU) patients in ZUHs. A further aim was to assess the genetic relatedness between the isolates of the most prevalent species among this group, considering this a primary step for further epidemiologic studies.

MATERIALS AND METHODS

Patient selection and collection of samples

A cross-sectional study was conducted in the Medical Microbiology and Immunology Department, Faculty of Medicine, Zagazig University, during the period from December 2014 to December 2015. One hundred and eighty patients (141 male and 39 female), who developed different infections at least 48 h after admission in either surgical or emergency ICU, were enrolled in this study. Their age ranged from one to 80 years(mean 33.4±15.6). A written informed consent was obtained from each patient or from their guardians before obtaining the samples. Demographic and clinical data of each patient was obtained through a worksheet filled for each case. Different non-repeated clinical samples (urine, endotracheal tube aspirate (ETA), pus and blood) were collected from patients according to the site of infection, using standard microbiologic methods. This study has been approved by the Institutional Review Board (IRB) of ZUHs.

Isolation and Identification

All samples were cultured on 5% blood agar and McConkey agar (Oxoid, England). Culture plates were incubated aerobically at 37° C for 48-72 h. Identification of non-fermenting colonies (NF) was primarily made by their reaction on triple sugar iron (TSI) medium (Oxoid, England), gram staining, oxidase test and was confirmed by API 20 NE (Bio-Mérieux, Marcy L´Etoile, France).

Antibiotic Susceptibility Tests

Isolates that were identified as being NFGNB, with the exclusion of *Pseudomonas* and *Acinetobacter* spp., were tested for their susceptibility to 14 different antimicrobials by disc diffusion method. Antibiotic discs included cefepime 30 µg, amikacin 30 µg, aztreonam 10 µg, tigecycline 15 µg, piperacillin/tazobactam (100/10) µg, meropenem 10 µg, colistin 10 µg, ceftazidime 30 µg, trimethoprim/sulphamethoxazole (1.25/23.75) µg, piperacillin 100 µg, imipenem 10 µg,

cefoperazone/sulbactam (75/30) µg, ciprofloxacin 5 µg and gentamycin 10 µg. All discs were purchased from Bioanalyse (TibbiMalzemelerSanayiveTicaret Ltd. Sti., Turkey) except tigecycline was from Oxoid, England. In addition, the MICs of ceftazidime, meropenem and levofloxacin (Sigma-Aldrich, St. Louis, USA) were determined by agar dilution method according to EUCAST[7] and CLSI guidelines [8].

Interpretation of the diameter of inhibition zones for meropenem, ceftazidime and TMP-SMX was done according to Kirby-Bauer zone diameter interpretative standardsas documented in CLSI**.** Those of cefoperazone-sulbactamwere interpreted according to the interpretative standards of *Enterobacteriaceae*. The results of other antibiotics were interpreted according to that of *Pseudomonas aeruginosa* (*P. aeruginosa*), as no interpretative standards for unusual NFGNB in concern to these antibiotics are yet available**.** Tigecycline inhibition zone diameters were interpreted according to FDA [9]. Interpretation of agar dilution tests was done according to CLSI guidelines. $MIC_{50}$and MIC_{90} values were calculated according to Schwartz et al. [10]. In all antibiotic susceptibility tests, *P. aeruginosa* ATCC® 27853 (Global Bioresource, Center of American Type Culture Collection KWIK-STIK ™) served as a quality control strain.

Random Amplified Polymorphic DNA (RAPD) Genotyping

The isolates belonging to the most prevalent species (*Burkholderiacepacia*complex [*Bcc*]), were genotyped by RAPD technique. *Bcc* isolates that were identified with accuracy of less than 99% by API 20NE were subjected to PCR reaction using 16S rDNA primers as described previously [11] to ensure their identity. RAPD genotyping was achieved by extracting DNA from the obtained *Bcc* isolates using QIAamp DNA Mini Kit (QIAGEN GmbH, Germany) according to the manufacturer's instructions, followed by RAPD PCR fingerprinting using the primer RAPD-270 (5′-TGC GCG CGG G-3′) and the cycling conditions described previously [12]. Reactions were performed in 20 µl reaction mixtures using Maxime PCR PreMix Kit (*i*-Taq) PCR beads (iNtRON Biotechnology, Korea). For each reaction, twoµl of extracted DNA and 40 µM of the mentioned primer (LGC, Biosearch Technologies, USA) were added. Amplification reactions were carried out using Veriti 96-well thermal cycler (Applied Biosystems, Singapore). PCR products were examined after electrophoresis in 1% agarose gel and visualized under UV light. Product sizes were determined using 50 bp DNA ladder (GeneOn, Germany). Interpretation of the fragments resulting from RAPD reaction was carried out by calculating the similarity index [13]. Then the similarity matrix data was subjected to cluster analysis with PAST (paleontological statistics) software [14] using unweighed pair group method for arithmetic average (UPGMA) to generate a dendrogram.

Statistical analysis

Data was analyzed using Statistical Package for the Social Sciences (SPSS version 20.0) software for analysis. Chi-square (χ^2) test and Student t-test were used to compare two qualitative and quantitative groups, respectively. Kruskal-Wallis test, a nonparametric test, was used to compare more than two groups.Kappa agreement was used to test the agreement level between two tests. P value of < 0.05 was considered significant.

RESULTS

Out of 516 cultivated clinical samples, 97 isolates (18.7%) were identified as being NFGNB. Among them, *P. aeruginosa* (44/97) had the highest isolation frequency (45.4%) followed by *Acinetobacterbaumannii* (36/97, 37.1%) then *Bcc* (16/97, 16.5%) and finally *Burkholderia pseudomallei* (*B. pseudomallei*) which had the lowest isolation frequency (only one isolate, 1.03%). After the exclusion of both *Pseudomonas* spp. and *Acinetobacter* spp., it was shown that unusual NFGNB were isolated from 3.3% (17/516) of all tested specimens, accounting for 17.5% (17/97) of all isolated NFGNB where *Bcc* isolates were the most prevalent (n=16) accounting for 94.1%, followed by *B. pseudomallei*(n=1) accounting for 5.9%.

Out of the 17 unusual NFGNB isolates, 11 isolates (64.7%) were obtained from urine specimens taken from catheterized patients. The remaining isolates (35.3%) were obtained from ETA specimens. No isolate was obtained from either blood or pus samples. The only isolate of *B. pseudomallei* was obtained from urine sample, while 62.5% of *Bcc* isolates were from urine samples and 37.5% were from ETA.

Nearly half of the patients (9/17 or 52.9%) who yielded positive culture for unusual NFGNB were below 20 years old (mean 23.4±13.3), while no isolates were obtained from patients between 60 and 80 years old. Most of unusual NFGNB isolates (15/17 or 88.2%) were obtained from male patients (88.2%) **(Table1)**, though this was insignificant statistically when analyzed using Chi-square test (P= 0.29). Regarding *Bcc* isolates, 93.75% were obtained from

male patients, the mean duration of ICU stay was 7.4±3.8 and prior antibiotic administration was observed in 62.5% of cases (10/16) **(Table 1)**.

TABLE (1): DEMOGRAPHIC AND CLINICAL DATA OF ICU PATIENTS FROM WHOM UNUSUAL NFGNB ISOLATES WERE OBTAINED

	Age (years)	Gender	Type of infection	Ward	Length of hospitalization (days)	Urinary catheter	Ventilator	Antibiotic use
1	22	Male	Urinary	Emergency ICU	5	√	√	√
2	15	Male	Respiratory	Emergency ICU	4	√	√	-
3	32	Male	Urinary	Emergency ICU	6	√	√	-
4	13	Male	Urinary	Emergency ICU	11	√	√	√
5	6	Male	Urinary	Emergency ICU	4	√	√	√
6	50	Male	Urinary	Surgery ICU	3	√	√	√
7	17	Male	Respiratory	Surgery ICU	15	√	√	√
8	47	Male	Urinary	Surgery ICU	5	√	√	√
9	16	Male	Urinary	Emergency ICU	3	√	√	√
10	32	Male	Urinary	Emergency ICU	7	√	√	-
11	37	Female	Respiratory	Surgery ICU	7	√	√	-
12	5	Male	Urinary	Emergency ICU	6	√	√	√
13	30	Male	Respiratory	Emergency ICU	4	√	√	-
14	12	Male	Respiratory	Emergency ICU	5	√	√	-
15	15	Male	Urinary	Emergency ICU	10	√	√	√
16	18	Male	Respiratory	Surgery ICU	16	√	√	√
17	30	Female	Urinary	Surgery ICU	3	√	√	-

Isolates 1-16 represent *Bcc* isolates, isolate 17 represents *B. pseudomallei*

Unfortunately, the only isolate of *B. pseudomallei* was lost during preservation. For this reason, only *Bcc* isolates were subjected to antibiotic susceptibility tests. The highest susceptibility ratio in disc diffusion method (68.8%) was recorded to tigecycline followed by ciprofloxacin (62.5%). On the other hand, no isolates were sensitive to ceftazidime, cefepime or piperacillin **(Figure 1)**.

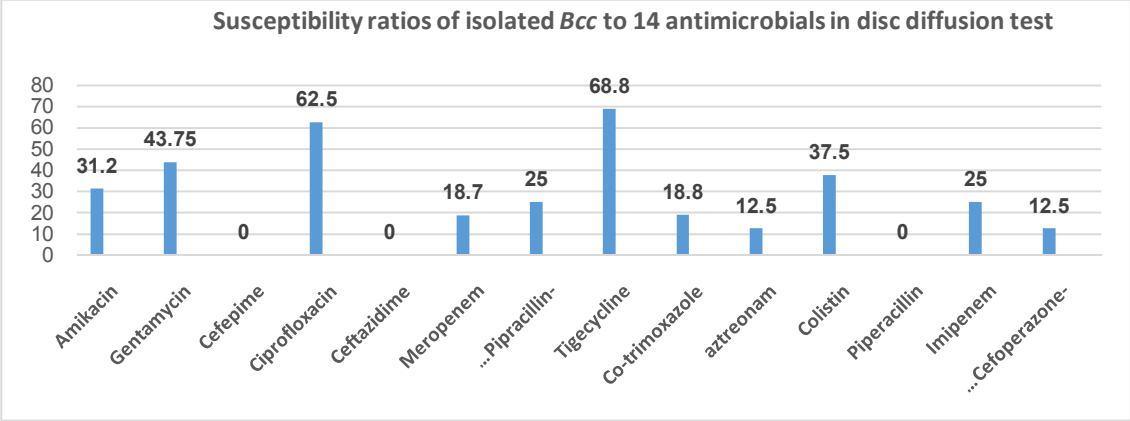

FIGURE (1): ANTIBIOTIC SUSCEPTIBILITY OF *BCC* ISOLATES BY DISC DIFFUSION METHOD. ISOLATES THAT HAD INTERMEDIATE SUSCEPTIBILITY WERE CONSIDERED RESISTANT IN THIS CHART. THE HIGHEST SUSCEPTIBILITY WAS RECORDED WITH TIGECYCLINE. NO ISOLATES WERE SUSCEPTIBLE TO CEFEPIME, CEFTAZIDIME OR PIPERACILLIN.

The MIC values of ceftazidime, levofloxacin and meropenem are presented in **Table 2**. According to CLSI 2015 guidelines, it was found that 62.5% of *Bcc* isolates were susceptible to ceftazidime with 6.25% of strains having intermediate susceptibility, 75% were susceptible to levofloxacin, and 50% were susceptible

to meropenem. When the MIC_{50} and MIC_{90} values for the three antimicrobials were calculated, those of ceftazidime were found to be 8 µg/ml and 512 µg/ml, respectively. Regarding levofloxacin, the values were 1 µg/ml and 32µg/ml, respectively. Finally, the values recorded for meropenem were 4 µg/ml and 128 µg/ml, respectively.

TABLE (2): CEFTAZIDIME, LEVOFLOXACIN AND MEROPENEM MIC VALUES (µg/ml) FOR *BCC* ISOLATES (N=16) BY AGAR DILUTION METHOD

Antibiotic	**Ceftazidime**			**Levofloxacin**			**Meropenem**		
***Bcc* isolates**	**S ≤8µg/ml**	**I =16µg/ml**	**R ≥32µg/ml**	**S ≤2µg/ml**	**I =4µg/ml**	**R ≥8µg/ml**	**S ≤4µg/ml**	**I =8µg/ml**	**R ≥16µg/ml**
1		16		2			0.015		
2	0.25			0.03			0.5		
3	8			0.25			0.125		
4	8			0.5					128
5	4			1					128
6			32	2			2		
7			512			32			128
8			512			8			32
9	8			1			4		
10	8			0.25			0.125		
11	4			2			2		
12	4			1					128
13			64			32			32
14	0.25			0.03			0.5		
15	8			1					128
16			512			32			128
P. aeruginosa ATCC ® 27853	1			4			0.25		
Median (range)	8 (0.25-512)			1 (0.03-32)			18 (0.015-128)		
Kruskal Wallis	6.45								
P	0.04*								

S; susceptible, I; intermediate, R; resistant,*significant.

Considering agar dilution as the reference method, good agreement (Kappa 0.5, P 0.001) was found between disc diffusion and agar dilution concerning the results of meropenem antibiotic. For ceftazidime antibiotic, the level of agreement could not be analyzed, as all *Bcc* isolates were resistant to it in the disc diffusion method **(Table 3)**. Levofloxacin antibiotic was not tested by disc diffusion method. For this reason, it was not included in this comparison.

TABLE (3): LEVEL OF AGREEMENT BETWEEN DISC DIFFUSION AND MIC RESULTS BY AGAR DILUTION (MIC) OF *BCC* ISOLATES (N=16) FOR CEFTAZIDIME AND MEROPENEM ANTIMICROBIALS

Antibiotic	**Ceftazidime**		**Meropenem**	
Isolate	**Disc**	**MIC**	**Disc**	**MIC**
1	R	I	R	S
2	R	S	S	S
3	R	S	I	S
4	R	S	R	R
5	R	S	R	R
6	R	R	I	S
7	R	R	R	R
8	R	R	R	R
9	R	S	S	S
10	R	S	I	S
11	R	S	I	S
12	R	S	R	R
13	R	R	R	R
14	R	S	S	S
15	R	S	R	R
16	R	R	R	R
X^2	NA		12.44	
P	-------		**0.002***	
Kappa agreement	NA*		0.50	
P	-------		**0.001****	
Disc sensitivity for detection of sensitive	0.0%		37.0%	
Disc specificity for detection of resistant	100.0%		100.0%	

R; RESISTANT, S; SUSCEPTIBLE, I; INTERMEDIATE SUSCEPTIBILITY, NA; NOT APPLICABLE, * SIGNIFICANT, **HIGHLY SIGNIFICANT

Following RAPD analysis of the obtained *Bcc* isolates **(Figure 2)**, the similarity indices ranged from zero to 0.66 **(Table 4)**. The resulting dendrogram demonstrated the presence of two different genetic clusters with different subclusters. It also demonstrated that the clusters are placed far from each other indicating the genetic diversity among the tested strains **(Figure 3)**.

L 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15

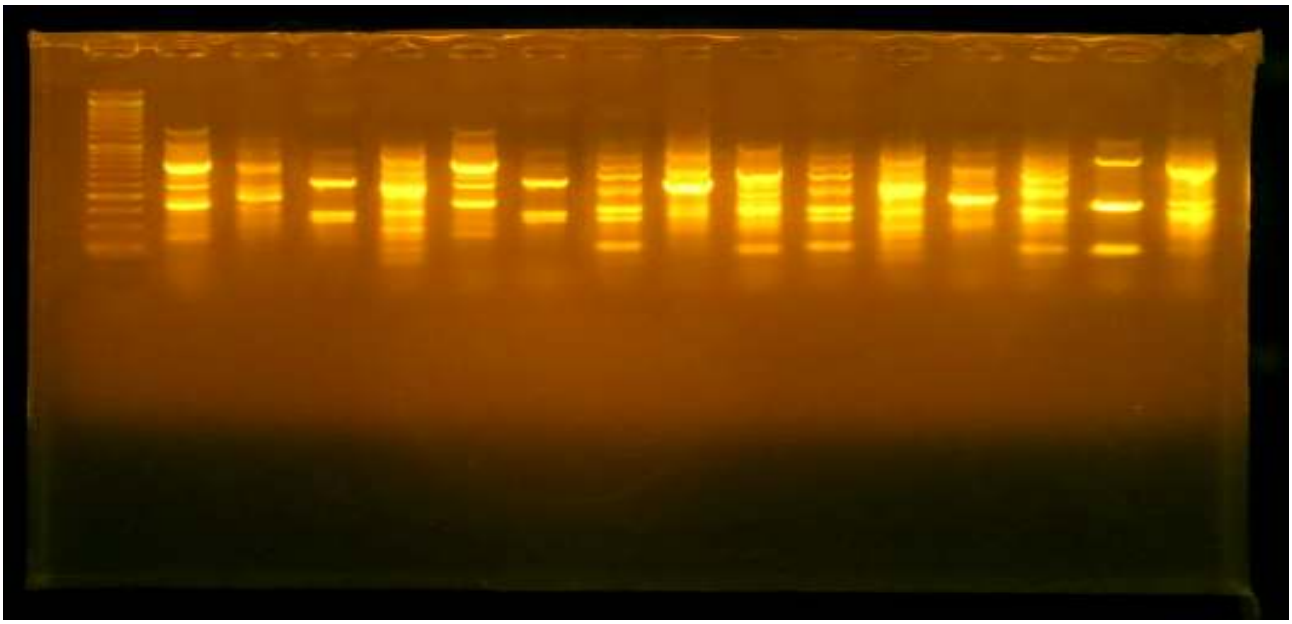

FIGURE (2): ELECTROPHORESIS GEL PHOTO DEMONSTRATING RAPD ANALYSIS RESULTS OF 15 *BCC* ISOLATES. LANE 1 (L) HAS 50-BP LADDER AND LANES 2-16 DEMONSTRATE RAPD RESULTS OF 15 *BCC* ISOLATES ARRANGED FROM 1 TO 15, THE SIXTEENTH ISOLATE IS NOT INCLUDED IN THIS PHOTO.

TABLE (4): JACCARD'S SIMILARITY COEFFICIENT (SIMILARITY INDEX) OF THE OBTAINED *BCC* ISOLATES BY RAPD ANALYSIS

	*Bcc*1	*Bcc*2	*Bcc*3	*Bcc*4	*Bcc*5	*Bcc*6	*Bcc*7	*Bcc*8	*Bcc*9	*Bcc*10	*Bcc*11	*Bcc*12	*Bcc*13	*Bcc*14	*Bcc*15	*Bcc*16
*Bcc*1	1															
*Bcc*2	0	1														
*Bcc*3	0	0	1													
*Bcc*4	0	0	0	1												
*Bcc*5	0	0.28	0	0.4	1											
*Bcc*6	0	0	**0.66**	0	0	1										
*Bcc*7	0.18	0.2	0	0.18	0	0.22	1									
*Bcc*8	0	0.28	0	0	0.25	0.29	0.2	1								
*Bcc*9	0	0	0	0.2	0	0.29	**0.6**	0.25	1							
*Bcc*10	0	0.2	0	0.18	0.2	0	0.33	0	0.2	1						
*Bcc*11	0	0	0.25	**0.6**	0.4	0	0	0	0	0.54	1					
*Bcc*12	0	0	0.25	0	0	0	0	0.2	0	0.36	0.4	1				

*Bcc*13	0	0	0.36	0.15	0	0.18	0.29	0.16	0.5	**0.14**	0.15	0.31	1			
*Bcc*14	0.22	0	0	0.25	0.57	0	0	0	0	0	0.25	0	0	1		
*Bcc*15	0.2	0	0	0.2	0	0	0	0.25	0	0.2	0.2	0.4	0.17	0.29	1	
*Bcc*16	0.2	0	0.29	0	0	0	0	0	0	0.2	0.2	0.2	0.17	0	0	1

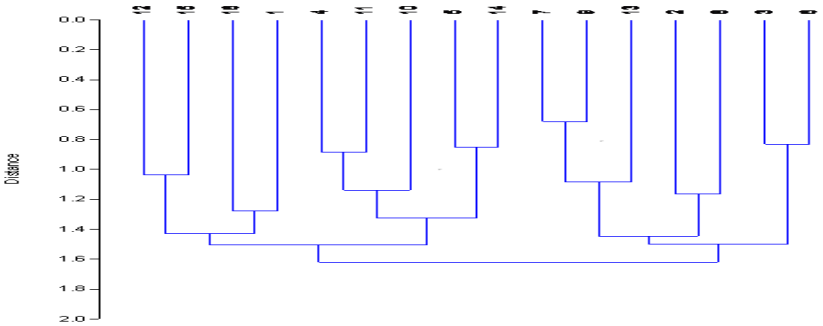

FIGURE (3):DENDROGRAM OF THE OBTAINED *BCC* ISOLATES AFTER ANALYSIS USING PAIRED GROUP METHOD. NO SINGLE ROOT IS DETECTED. IT ENDS IN TWO MAJOR CLUSTERS WITH SUBCLUSTERS THAT ARE PLACED FAR FROM EACH OTHER.

DISCUSSION

The isolation rate of NFGNB (18.7%) recorded in our study comes in agreement witha previous Indian study carried out in a tertiary care hospital, where it was 16.18% [15].Whereas, it comes higher than that recorded in another Indian tertiary care hospital where it was 9.32% [16].

The unusual NFGNB accounted for 3.3% isolation frequency among all tested specimens that comes much similar to what was reported previously among respiratory tract infection in an Indian tertiary care hospital (4%) [17]. *Bcc* was the most prevalent species accounting for16.5% of the total NFGNB. This comes much higher than what was reported previously where *Bcc* accounted for 4.66% of all isolated non-fermenters in an Iranian study [18]. Lower ratios were also reported in other countries(4.58% and 12.1%) [15] [17]. On the other hand, our result is lower than that recorded in Latin America, where it accounted for 47.15% among all NFGNB (83/176) in a surveillance study that lasted from 1997 to 2002 [19]. While *Bcc* represented the main unusual NFGNB (16/17, 94.1%) in the current study, *Stenotrophomonas maltophilia* (*S. maltophilia*) was the most frequently isolated species among unusual NFGNB in India (45.5%) [17] as well as in Saudi Arabia (20.8%) [6]. The different identification system used in the previous two studies (Vitek 2 system) compared to API 20NE used in the current study may have contributed to this difference.

Only one isolate of *B. pseudomallei*was obtained accounting for 5.9% of the unusual NFGNB and 1.03% of the total NFGNB. Similar results were reported in a previous Indian study where two isolates among 33 unusual NFGNB were *B. pseudomallei* accounting for 6.1% of unusual NFGNB isolated from patients with nosocomial pneumonia [17].

Urine was the main source of isolation of *Bcc* (62.5%) followed by ETA (37.5%). This comes in contrast to the general belief that *Bcc* is a cause of chronic respiratory infection or colonization particularly in cystic fibrosis (CF) patients [20]. In a Turkish study, ETA was the main source for *Bcc*isolation(58.9%) [21]. However, 62.7% of *Bcc* were isolated from blood specimens in another study [19] and in a previous Egyptian study, the highest percent (85.7%) of *Bcc* isolates were from pus specimens [22]. This difference could be attributed to the difference in the clinical conditions of patients along with different hospital wards selected in each study.

The relatively short ICU stay for patients from whom *Bcc* isolates were isolated (mean 7.4 ± 3.8 days), in our study, was an unusual finding. In a previous Turkish study, the researchers reported that the mean duration of hospitalization for *Bcc* infected patients was 15.2 ± 9.9 days [21]. This may, in some way, point to lower compliance to infection control measures that may result in a more rapid infection.

Our study revealed that prior antibiotic use was observed in 62.5% of patients positive for *Bcc* isolates.

Prior antibiotic administration in 38.5% of *Bcc* infected patients was previously reported [21]. This difference may be explained by the different treatment policies concerning antimicrobials in different hospitals. Besides, the researchers of the previous study conducted their work in different hospital wards, whereas, we focused on ICU patients where excessive use of antimicrobials is confronted.

*B. cepacia*complex bacteria are well known for their multidrug resistance. Both intrinsic and acquired mechanisms contribute to this phenomenon [23] [24]. In the current study, the antimicrobial susceptibility pattern of the sixteen *Bcc* isolates was tested by disc diffusion and agar dilution methods. In disc diffusion, the highest susceptibility rate was observed for tigecycline (68.8%) and ciprofloxacin (62.5%). This was followed by gentamycin (43.75%), colistin (37.5%), amikacin (31.2%), then piperacillin-tazobactam and imipenem (25% each), co-trimoxazole (18.8%), meropenem (18.7%) then both cefoperazone-sulbactam and aztreonam (12.5% each). On the other hand, all isolates were resistant to ceftazidime and piperacillin.Different patterns were recorded in previous studies [15] [21] [22] [25]but the striking point, in our results, is the high level of ceftazidime resistance (100%) which was not recorded previously, as far as we know. This could be attributed to the excess use of 3rd generation cephalosporins in our hospital. This clearly highlights the importance of local susceptibility tests that should guide treatment policies.

The agar dilution method demonstrated that 62.5%, 75% and 50% of *Bcc* isolates were susceptible to ceftazidime, levofloxacin and meropenem, respectively. Varying results were obtained in previous studies [19] [21] [26] [27]. All of them recorded *Bcc* as one of the highly resistant microbes encountered. In spite of this, a more recent study reported that all *Bcc* isolated from CF children (*B. cepacia*, *B. cenocepacia*, and *B. multivorans*) were susceptible to levofloxacin, ceftazidime, and meropenem[28]. This indeed confirms the different behavior of *Bcc* isolates in susceptibility tests and points to the unpredictable nature of their results.

Our study recorded higher MIC_{90} values for the tested three antimicrobials, compared to previous works [19] [26]. This reflects the higher number of resistant *Bcc* isolates obtained from our hospital compared to the other studies. A significant agreement (P 0.001) between disc diffusion and agar dilution methods for meropenem was found in our study. As all isolates were resistant to ceftazidime in disc diffusion, the level of agreement with agar dilution could not be assessed, but this made the test 100% specific in detecting resistant isolates with no false susceptibility detected.

Being the first time to record the isolation of *Bcc* in our hospital,we tried further to assess the genetic relatedness of the obtained isolates to be a primary step towards more understanding of the spread of this organism. Our results demonstrated that *Bcc* isolates obtained from patients in surgery and emergency ICUs, belonged to two genetic clusters which have further subclusters and which are genetically distinct from each other. To date, most of the previous studies performed genotyping for *Bcc* obtained from CF patients with their results being contradicting [29] [30].

CONCLUSION

In conclusion, this study demonstrated that *Bcc* constituted an emerging nosocomial pathogen in ICUs of ZUHs with high resistance to different antimicrobials. So far, the problem is not great. However, this necessitates further studies that continuously monitor its occurrence and that assess possible sources of infection among ICU patients as well as other hospital units.

CONFLICT OF INTEREST

The authors declare no conflict of interest

REFERENCES

[1] **Quinn JP.**Clinical problems posed by multiresistantnonfermenting gram-negative pathogens. Clin. Infect. Dis. 1998; 27(Suppl 1):S117-124.http://www.ncbi.nlm.nih.gov/pubmed/9710680

[2] **Enoch DA, Birkett CI and Ludlam HA.** Non-fermentative gram-negative bacteria. Int. J. Antimicrob. Agents 2007; 29:S33–S41.http://www.ncbi.nlm.nih.gov/pubmed/17659210

[3] **Vidal F, Mensa J, Almela M, Olona M, Martínez JA, Marco F, López MJ, Soriano A, Horcajada JP, Gatell JM and Richart C.**Bacteraemia in adults due to glucose nonfermentativegram-negative bacilli other than *Pseudomonas aeruginosa*. QJM2003; 96:227-234.http://www.ncbi.nlm.nih.gov/pubmed/12615987

[4] **Singhal T, Shah S and NaikR.**Outbreak of *Burkholderiacepacia* complex bacteremia in a chemotherapy day care unit due to intrinsic contamination of an antiemetic drug.Ind. J.Med.Microbiol. 2015; 33, 117-119.http://scicurve.com/paper/25560013

[5] **Jacquier H, Carbonnelle E, Corvec S, Illiaquer M, Le Monnier A, Bille E, Zahar JR, Beretti JL, Jauréguy F, FihmanV, Tankovic J and CattoirV.**Revisited distribution of

non-fermenting gram-negative bacilli clinical isolates. Europ. J. Clin. Microbiol. Infect. Dis. 2011; 30:1579-1586.http://www.ncbi.nlm.nih.gov/pubmed/21509476

[6] **Asaad AM, Al-Ayed MZ and Qureshi MA.**Emergence of unusual non-fermenting gram-negative nosocomial pathogens in a Saudi hospital. Jap. J. Infect. Dis.2013; 66:507-511.http://www.ncbi.nlm.nih.gov/pubmed/24270139

[7] **European Committee for Antimicrobial Susceptibility Testing (EUCAST) of the European Society of Clinical Microbiology and Infectious Dieases (ESCMID).** EUCAST Definitive Document E.DEF 3.1, June 2000: Determination of minimum inhibitory concentrations (MICs) of antibacterial agents by agar dilution. Clin. Microbiol. Infect. 2000; 6: 509-515.http://www.ncbi.nlm.nih.gov/pubmed/11168187

[8] **Clinical Laboratory Standards Institute.** Performance Standards for Antimicrobial Susceptibility Testing; twenty fifth Informational Supplement M100-S25 Vol. 35 No. 3. Clinical and Laboratory Standards Institute Wayne, Pa., USA.
http://shop.clsi.org/site/Sample_pdf/M100S25_sample.pdf

[9] **Food and Drug Administration (FDA)**, 2010 (http://www.accessdata.fda.gov/Scripts/cder/DrugsatFDA/). Accessed March 9, 2016.

[10] **Schwartz S, Silley P, Simjee S, Woodford N, van Duijkeren E, Johnson AP and GaastraW.**Assessing the antimicrobial susceptibility of bacteria obtained from animals. Vet. Microbiol. 2010; 141:1-4.http://www.ncbi.nlm.nih.gov/pubmed/20042302

[11] **Mahenthiralingam E, Bischof J, Byrne SK, Radomski C, Davies JE, AV-Gay Y andVandamme P.**DNA- based diagnostic approaches for identification of *Burkholderiacepacia* complex, *Burkholderiavietnamiensis*, *Burkholderiamultivorans*, *Burkholderiastabilis*, and *Burkholderiacepacia*genomovars I and III. J. Clin. Microbiol. 2000; 38:3165-3173.http://www.ncbi.nlm.nih.gov/pubmed/10970351

[12] **Coenye T, Spilker T, Martin A and LiPumaJJ.**Comparative assessment of genotyping methods for epidemiologic study of *Burkholderiacepacia*genomovar III. J. Clin. Microbiol. 2002; 40:3300-3307. http://www.ncbi.nlm.nih.gov/pubmed/12202570

[13] **Tilwari A, Chouhan D and Sharma R.**Random amplified polymorphic DNA (RAPD) analysis of microbial community diversity in soil affected by industrial pollutants: reference to Mandideep industrial area. Afr. J Microbiol. Res. 2013;**7**:3933-3942.http://www.academicjournals.org/journal/AJMR/article-full-text-pdf/4F4CA5E12772

[14] **Hammer Ø, Harper DAT and Ryan PD.**PAST: Paleontological Statistics Software Package for Education and Data Analysis. Palaeontologia Electronica 2001; 4:9 pp.http://palaeo-electronica.org/2001_1/past/past.pdf

[15] **Nautiyal S, Jauhari S, Goel N andMahawalBS.**Current trend of non-fermenting gram-negative bacilli in a tertiary care hospital in Dehradun, Uttarakhand. Int. J. Adv. Res. 2014; 2:322-328.file:///C:/Users/ameer/Downloads/849_IJAR-2694%20(1).pdf

[16] **Juyal D, Prakash R, Shganakarnarayan SA, Sharma M, Negi V and Sharma N.** Prevalence of non-fermenting gram-negative bacilli and their *in vitro* susceptibility patterns in a tertiary care hospital of Uttarakhand: a study from foothills of Himalayas. Saudi Journal ofHealth Sciences 2013; 2:108-112.http://www.saudijhealthsci.org/article.asp?issn=2278-0521;year=2013;volume=2;issue=2;spage=108;epage=112;aulast=Juyal

[17] **Chawla K, Vishwanath S and Munium FC.** Non-fermenting gram-negative bacilli other than *Pseudomonas aeruginosa* and *Acinetobacterbaumunii*causing respiratory tract infections in a tertiary care center. J. Glob. Infect. Dis. 2013; 5:144-148.http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3958983

[18] **Rahbar M, Mehragan H andAkbariNH.**Prevalence of drug resistance innonfermenter gram-negative bacilli. Iran. J. Pathol. 2010; 5:90-96.http://www.sid.ir/en/VEWSSID/J_pdf/110920100207.pdf

[19] **Gales AC, Jones RN, Andrade SS andSader HS.** Antimicrobial susceptibility patterns of unusual nonfermentative gram-negative bacilli isolated from Latin America: report from the SENTRY Antimicrobial Surveillance Program (1997–2002). Mem. Inst. Oswaldo Cruz 2005; 100:571–577.http://www.ncbi.nlm.nih.gov/pubmed/16302068

[20] **Coutinho HD, Falcão-Silva VS and GonçalvesGF.**Pulmonary bacterial pathogens in cystic fibrosis patients and antibiotic therapy: a tool for the health workers. Int. Arch. Med. 2008; 1:24.http://www.ncbi.nlm.nih.gov/pubmed/18992146

[21] **Dizbay M, Tunccan O, Sezer B, Aktas F and Arman D.** Nosocomial *Burkholderiacepacia* infections in a Turkish University Hospital: a five-year surveillance. J. Infect. Dev. Ctries 2009; 3: 273–277.http://www.ncbi.nlm.nih.gov/pubmed/19759490

[22] **Omar N, Abd El Raouf H, Okasha H and Nabil N.** Microbiological assessment of *Burkholderiacepacia* complex (*BCC*) isolates in Alexandria Main University Hospital. Alexandria Journal of Medicine 2015; 1: 41-46.http://www.sciencedirect.com/science/article/pii/S2090506814000852

[23] **SchweizerHP.**Mechanisms of antibiotic resistance in *Burkholderiapseudomallei*: implications for treatment of melioidosis. Future Microbiol. 2012;**7**:1389–1399.http://www.ncbi.nlm.nih.gov/pubmed/23231488

[24]**Papp-Wallace KM, Taracila MA, Gatta JA, Ohuchi N, Bonomo RA andNukagaM.**Insights into beta-lactamases from *Burkholderia* species, two phylogenetically related yet distinct resistance determinants. The Journal of Biological Chemistry 2013; 288:19090-19102.http://www.ncbi.nlm.nih.gov/pubmed/23658015

[25] **Gautam V, Vandamme P, Ray P, Chatterjee SS, Das A, Sharma K,Rana S, Garg RK, Madhup SK, Mahajan M and Sharma M.**Identification of lysine positive non-fermenting gram-negative bacilli (*Stenotrophomonasmaltophilia* and *Burkholderiacepacia* complex).Ind. J. Med. Microbiol. 2009; 27:128-133.http://www.ncbi.nlm.nih.gov/pubmed/19384035

[26] **Bonacorsi S, Fitoussi F, LhopitalSandBingenE.**Comparative*in vitro* activities of meropenem, imipenem, temocillinpiperacillin, and ceftazidime in combination with tobramycin, rifampin, or ciprofloxacin against *Burkholderiacepacia* isolates from patients with cystic fibrosis. Antimicrob. Agents Chemother.

1999; 43:213–217 .http://www.ncbi.nlm.nih.gov/pubmed/9925508
[27] **Gautam V, Kumar S, Kaur P, Deepak T, Singhal L, Tewari R and Ray P.**Antimicrobial susceptibility pattern of *Burkholderiacepacia* complex &*Stenotrophomonasmaltophilia* over six years (2007-2012). Ind. J. Med. Res. 2015; 142:492-494.http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4683836/
[28] **Vicenzi, FJ, Pillonetto M, Aguilar H, de Souza PHdM, Palmeiro JK, Riedi CA, Rosario-Filho NA and Dalla-Costa LM.**Polyphasiccharacterization of *Burkholderiacepacia*complex species isolated from children with cystic fibrosis. Mem. Instit. Oswaldo Cruz 2016; 111:37-42.http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4727434/
[29] **Mahenthiralingam E, Campbell ME, Henry DA and SpeertDP.**Epidemiology of *Burkholderiacepacia* infection in patients with cystic fibrosis: analysis by randomly amplified polymorphic DNA fingerprinting. J.Clin. Microbiol. 1996; 34:2914–2920.http://www.ncbi.nlm.nih.gov/pubmed/8940422
[30] **Detsika MG, Corkill JE, Magalhães M, Glendinning KJ, Hart CAandWinstanley C.** Molecular typing of, and distribution of genetic markers among, *Burkholderiacepacia* complex isolates from Brazil. J. Clin.Microbiol. 2003; 41:4148–4153.http://www.ncbi.nlm.nih.gov/pmc/articles/PMC193793/