www.jmscr.igmpublication.org
Impact Factor 5.84
Index Copernicus Value: 71.58
ISSN (e)-2347-176x ISSN (p) 2455-0450
crossref DOI: https://dx.doi.org/10.18535/jmscr/v5i12.28

IGM Publication
Journal Of Medical Science And Clinical Research
An Official Publication Of IGM Publication

Existence of Burkholderia cepacia at Burn and Intensive Care Units in Alexandria Main University Hospital, Egypt

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Abstract
Nosocomial spread of B. cepacia complex (Bcc) isolates amongst non- cystic fibrosis (CF) patients especially immunocompromised patients has been documented, where inadequate laboratory identification and limited treatment options are considered the main obstacles hindering accurate diagnosis and thus proper therapeutic outcome. The present study aimed to detect the isolation percentage of BCC from patients who admitted to ICUs and burn units of Alexandria Main University Hospital during 6 months from April to October 2015. A total of 150 clinical samples were collected from these patients. All samples were cultured and subjected to microbiological procedures for isolation and identification of isolates. Out of 150 clinical samples cultured, 128(85.3%) showed isolates and 22(14.7%) no growth. The majority of isolates causing burn wound infection were P.aeruginosa (31.0%), followed by B.cepacia (30.0%), E.coli (17.0%) and S.aureus (MRSA) (10.0%). BCC was further identified by the BSCA and PCR system (rec A gene). Antibiotic susceptibility tests revealed that all Bcc isolated were Multi Drug Resistant (MDR), the lowest percentage of BCC resistance was against Imipinem (IPM) and Meropenem (MEM)(50% each).
Abbreviations: *Bcc, Burkholderia cepacia complex; CF, cystic fibrosis; ICUs, Intensive care units; MDR, Multi Drug Resistant; NFGNB, nosocomial non-fermentative Gram negative bacilli; BCSA, B. cepacia selective agar.*
Keywords: *Burkholderia cepacia complex; Identification; Antimicrobial susceptibility.*

Introduction

B. cepacia complex is well recognized as a significant pathogen associated with colonization and pulmonary infection in cystic fibrosis (CF) patients. However, the pathogenicity of Bcc is not always limited to CF or immunocompromised patients. Several surveys now report the increasing or simultaneous persistence of Pseudomonas aeruginosa, Acinetobacterspp. And Stenotrophomonas maltophilia nosocomial infections. As well as the emergence of newer nosocomial non-fermentative gram negative bacilli (NFGNB) such as Bcc that causes serious problems in clinical settings because of its high transmissibility between hospitalized patients and its multiple drug resistance (MDR).[(1-3)] It is associated with a wide variety of infections among hospitalized patients who are

usually infected via contaminated equipment or exposure to contaminated solutions. The infections usually include pneumonia, bacteremia, skin and soft tissue infection and genitourinary tract infection.(4-6)

Generally, in the literature reports of nosocomial B. cepacia infections are usually restricted to nosocomial epidemics or outbreaks. Reports of sporadic cases of B. cepacia nosocomial infections are rare, probably due to the lack of specific laboratory tests in routine testing in most hospitals, so B. cepacia has been ambiguously reported as NFGNB or simply Pseudomonas spp. This was the case in the routine microbiology laboratory before this study. This also explains the lack of reports about the prevalence of B. cepacia infections in Egypt and many countries.(7-9)

In routine clinical laboratories, the identification of putative Bcc isolates is generally performed using a combination of selective media, conventional biochemical analysis and/or commercial systems. Several different media have been developed for the selective isolation of Bcc isolates from different specimens(10-12) such as B. cepacia selective agar (BCSA), Pseudomonas cepacia (PC) agar, or oxidation-fermentation polymyxin-bacitracin lactose (OFPBL) agar.(13)

BCSA is more enriched than OFPBL or PCA, where yeast extract and casein provide a rich variety of ingredients that overcome the nutritional deficiencies which may prevent some strains of B. cepacia from growth on other selective media. Organisms not belonging to the Bcc that are capable of growth on BCSA include B. gladioli, Ralstonia spp. and Pandoraea spp.(14,15) It is also associated with a wide variety of infections among hospitalized patients who are usually infected via contaminated equipment during their hospitalization such as indwelling catheters, ventilators, urethral instrumentation or exposure to contaminated solutions including antiseptics, disinfectants, nebulizer fluids and dextrose solutions. The infections usually include pneumonia, bacteremia, skin and soft tissue infection and genitourinary tract infection.(15)

Bcc has intrinsic resistance and is one of the most antimicrobial-resistant organisms encountered. Therefore, it needs to be correctly identified and differentiated from P. aeruginosa as Bcc has inherently contrasting susceptibility pattern to that of P. aeruginosa. Bcc is intrinsically resistant to antimicrobial agents such as aminoglycosides, first- and second-generation cephalosporins, antipseud-omonal penicillins and polymyxins. Thus giving extreme value to the proper identification of B. cepacia.(9-16)

The present study aimed to detect the isolation percentage of Bcc from the patients were admitted to burn units and ICUs of the Alexandria Main University Hospital throughout a period of 6 months (April to October 2015) and to determine their antibiotic susceptibility pattern.

Methods

The present study was conducted on 115 patients admitted to burn units and ICUs in Alexandria Main University Hospital, Egypt, throughout a period of 6 months. A total of 150 clinical samples: (Mini BAL, urine, burn wound, tissue and blood) (Table: 1) were collected from patients showing signs and symptoms of infection after more than 48 hrs. of admission to hospital. A questionnaire sheet was filled for every patient included all relevant information (age, sex, clinical picture, treatment before and after admission etc...).

Collection and processing of samples

- The samples were aseptically collected, labeled and then transported to the lab. All samples were cultured on 5% blood agar and McConkey agar (Oxoid) Culture plates were incubated aerobically at 37° C for 24 hrs.

Isolation and identification of BCC:

All isolates on MacConkey's that were identified as Gram negative non-lactose fermenting colonies that were oxidase positive, and complete identification were done by biochemical reaction (Table :2) and suspected to be BCC were subcultured on BCSA, incubated at 37°C for 24-48 hours, examined after 48 hours. Typical colonies were circular and entire. Color formation is based on natural pigment

expression and colonies varied from orange to bright pink were BCC, while yellow pigment colonies were non BCC. Reference strain *of Pseudomonas aeruginosa* was also cultured on BCSA as negative control strain and the results were confirmed by PCR technique.

Antibiotic susceptibility testing

Identified BCC strains were screened for their antimicrobial susceptibility using disk diffusion method described by Bauer et al (). The test was done on Mueller–Hinton agar (Oxoid) plates were inoculated with a bacterial suspension equivalent to a 0.5 McFarland Standard, using the selected antibiotic susceptibility discs (Oxoid) with various concentrations including Ceftazidime (CAZ) 30 µg, Meropenem (MEM) 10 µg, Tetracycline (TE) 30 µg, Trimethoprim-Sulphamethoxazole (SXT) 1.25/23.75 µg, Tobramycin (TOB) 10 µg, Chloramphenicol (C) 30 µg, Piperacillin–Tazobactam (TZP) 100/10 µg, Ciprofloxacin (CIP) 30 µg. Zones of growth inhibition were measured after overnight incubation at 37oC and susceptibility was interpreted as susceptible(s), intermediate (I) and resistant (R) according to standard tables published by Clinical Laboratory Standard Institute (CLSI).

Detection of bacterial RECA gene by polymerase chain reaction (PCR):[17]

The test aimed to identify *B.cepacia* by the presence of bacterial recA gene in all clinical specimen.

Principle

The DNA was first extracted from samples, and then amplification was performed in presence of specific primers. The amplified DNA was analysed by the use of gel electrophoresis.

DNA extraction from samples

DNA was extracted from 32 *B.cepacia* isolates by boiling five colonies of fresh organism culture in 20 µl of sterile distilled water for 5 minutes, followed by centrifugation for 2 min at 8000 rpm, those supernatants were used for amplification.

Finally, the purified DNA was capped and stored at -20°C, till performing the PCR amplification.

Amplification reaction

Reagents

- **Master Mix** MyTaq™ Red Mix (bio 25043, bio 25044) to use solution containing nonrecombinant modified form of native Taq DNA polymerase, reaction buffer (pH 8.5), MgCl2 and dNTPs. It also contains two dyes (blue and yellow) that allow monitoring of progress during electrophoresis.
- **Primers**: the primers used for amplification detect a 1.040-bp region of the rec A gene. (Table: 3)

Primers were reconstituted with appropriate volumes of sterile distilled water as follows:

355 F was reconstituted by 815.80µl for 100µM, and 910 R was reconstituted by 784.85 µl for 100 µM, and then further diluted 1:10.

Protocol of Amplification: Reaction volumes (prepared on ice) contained the following components with a final volume of 25 µl each.

- 12.5 µl Master Mix
- 0.5 µl DNA extract
- 1 µl forward primer
- 1 µl reverse primer
- 10 µl distilled water

DNA detection

The amplification products were analysed by agarose gel electrophoresis and ethidium bromide staining. The DNA bands were visualized on a 320 nm UV transillumінator. The gel was examined for specific bands of 1.040 bp as determined by the molecular weight markers run at the same time.

Results

In a 6 months period, a total of 32 BCC strains were isolated from different clinical samples collected from burn and ICU patients admitted to Alexandria Main University Hospital. Out of 150 clinical samples cultured, 128(85.3%) showed isolates and 22(14.7%) no growth. The majority of isolates causing burn wound infection were P.aeruginosa (31.0%), followed by *B.cepacia* (30.0%), E.coli (17.0%) and S.aureus (MRSA) (10.0%). While the most of isolates causing infection in ICUs were

A.baumanii (19.8%) followed by S.aureus (MRSA) (18.2%) Table (4)

Table (1): Description of the clinical samples.

Type of sample	ICU	Burn unit	Total
Swabs from wound	2	69	71
Mini-BAL	31	0	31
Urine	27	2	29
Blood	13	2	15
Tissue	0	2	2
CSF	1	0	1
Pleural effusion	1	0	1
Total	75	75	150

Table (2): Differentiation of members of *B. cepacia* complex, *P. aeruginosa*, *Ralstonia spp.* and *Pandoraea spp.*

	B. cepacia complex	*P. aeruginosa*	*Pandoraea* spp	*Ralstonia* spp.
Oxidase	+	+	V	+
Growth on BCSA	+	-	+	+
Lysine Decarboxylase	+	-	-	-
Ornithine Decarboxylase	V	-	-	-
Motility	+	+	+	-

Table (3): Oligonucleotide used as primers for the detection and identification of B. cepacia complex genomovars in clinical samples by multiplex PCR assay.

Primer	Sequence (5′ to 3′)	Specificity
BCR1[a]	TGA CCG CCG AGA AGA GCA A	*recA* gene—*B. cepacia* complex
BCR2[a]	CTC TTC TTC GTC CAT CGC CTC	*recA* gene—*B. cepacia* complex

Table (4): Frequency of isolates from studied clinical samples in ICUs and burn units.

Isolated organisms	Burn units		ICUs		Total	
	No.	%	No.	%	No.	%
Gram negative bacilli:	**(86)**	**(86%)**	**(42)**	**(33.3%)**	**(128)**	**(56.6%)**
P. aeruginosa	31	31.0	12	9.5	43	19.0
B. cepacia	30	30.0	2	1.8	32	14.2
E.coli	17	17.0	7	5.5	24	10.6
K.pneumoniae	6	6.0	15	11.9	21	9.3
P. mirabilis	2	2.0	3	2.3	5	2.2
Enterobacter spp.	0	0.0	3	2.3	3	1.3
Gram negative coccobacilli:	**(2)**	**(2%)**	**(25)**	**(19.8%)**	**(27)**	**(11.9%)**
A. baumannii	2	2.0	25	19.8	27	11.9
Gram positive cocci:	**(10)**	**(10%)**	**(31)**	**(24.5%)**	**(41)**	**(18.1%)**
S.aureus (MRSA)	10	10.0	23	18.2	33	14.6
E.faecalis	0	0.0	8	6.3	8	3.5
Gram positive bacilli:	**(2)**	**(0.2%)**	**(4)**	**(3.2%)**	**6**	**(2.7%)**
Diphteroids	(2)	(0.2%)	(4)	(3.2%)	6	(2.7%)
Fungi (*Candida spp.*)	**0**	**(0.0%)**	**(24)**	**(19.2%)**	**24**	**(10.7%)**
Total	**100**	**100.0**	**126**	**100.0**	**226**	**100.0**

It is clear from table (4) and figure (1) that the distribution of isolated bacteria from burn units and ICUs. Out of 226 isolates were 100 (44.2%) isolated from burn units and 126 (55.8 %) were from ICUs. The majority of isolates from burn units were Gram negative bacilli 86 (86%), the most of them were P. aeruginosa (31.0%) and B. cepecia (30.0%), followed by Gram positive cocci were 10(10%) all of them were S.aureus (MRSA), while the lowest percentage from burn unit were A. baumannii (2.0%).

In ICUs, out of 126 isolates, 25(19.8%) were A.baumanii, 42(33.3%) were Gram negative bacilli, the highest percentage of them were K.pneumoniae 15(11.9%), P. aeruginosa 12(9.5%), E.coli 7(5.5%), followed by P. mirabilis and Enterobacter spp (2.3% each) and two isolates only for *B.cepacia* 2(1.8%),while Gram positive cocci showed (24.5%) of isolates , most of them were S.aureus (MRSA) 23(18.2%), and candida spp. (19.2%) respectively.

There were statistical significant difference between the distribution of *B.cepacia* from burn units and ICUs. ($p < 0.001$*)

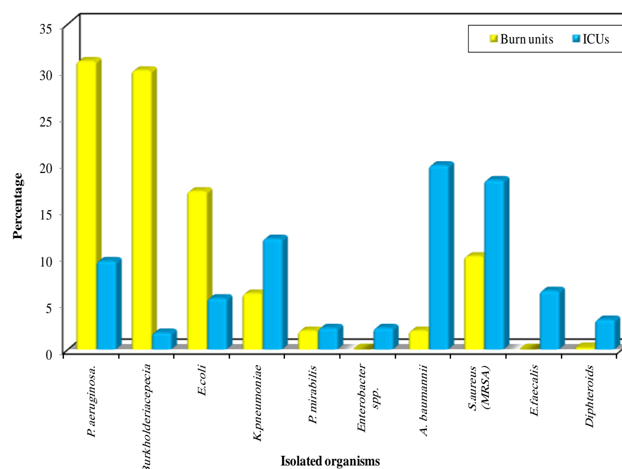

Figure (1): Frequency of isolates from studied clinical samples in ICUs and burn units

Table (5): Distribution of the 32 *B.cepacia* complex strains isolated from different clinical samples, Alexandria Main University Hospital.

Type of samples	No. of isolates	Result					
		Burn units		ICUs		Total	
		No	%	No	%	No	%
Burn wound	61	26	86.8	--	--	26	81.2
Mini-BAL	29	--	--	2	100.0	2	6.2
Urine	27	1	3.3	--	--	1	3.1
Blood	9	1	3.3	--	--	1	3.1
CSF	0	--	--	--	--	0	0.0
Pleural effusion	0	--	--	--	--	0	0.0
Tissue	2	2	6.6	--	--	2	6.2
Total	128	30	100.0	2	100.0	32	100

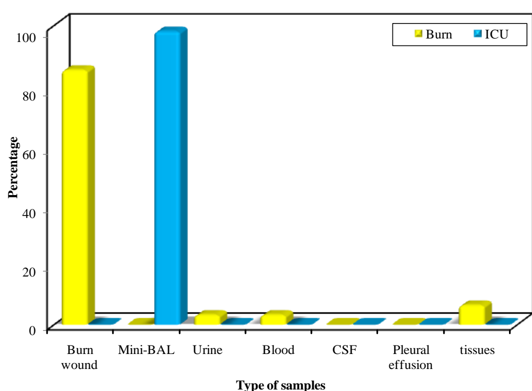

Figure (2): Distribution of the 32 *B.cepacia* complex strains isolated from different clinical samples, Alexandria Main University Hospital.

Table (6): Distribution of 30 *B.cepacia* isolates in burn units.

Time of sampling	Male dep.		Female dep.		Pediatric dep.		Total	
	No.	%	No.	%	No.	%	No.	%
1st week	2	13.3	0	0.0	1	10.0	3	10.0%
2nd week	3	20.0	1	20.0	2	20.0	6	20.0%
3rd week	4	26.7	1	20.0	2	20.0	7	23.3
4th week	6	40.0	3	60.0	5	50.0	14	46.7
Total	**15**	**100.0**	**5**	**100.0**	**10**	**100.0**	**30**	**100.0**

*Only two isolates from Mini-BAL specimen from two male patients were admitted to ICU due to RTA in the 4th week of admission.

The distribution of *B.cepacia* increased with the more duration of stay in burn units, where the infection in male department increased from 13.3% in the first week to 40.0% in the fourth week, while in female department increased from (0.0%) to (60.0). On the other hand, in pediatric department increased from (10.0%) to (50.0%). Table (6) and figure (3)

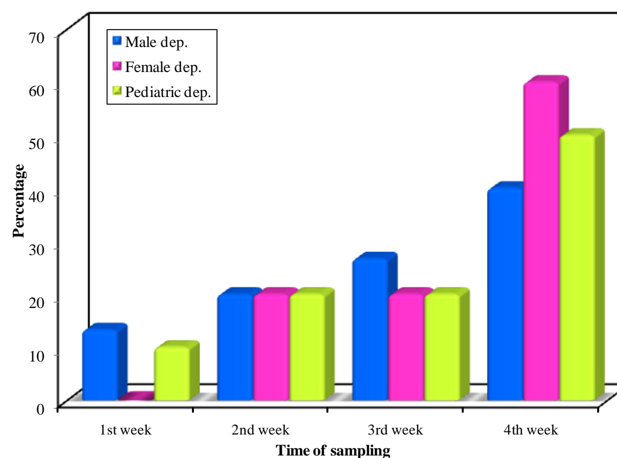

Figure (3): Distribution of 30 *B.cepacia* isolates in burn units

Table (7): Frequency of 30 *B.cepacia* isolates according to cause of burn.

Cause of burn	Male dep.		Female dep.		Pediatric dep.		Total	
	No.	%	No.	%	No.	%	No.	%
Flame burn	4	26.7	3	60.0	1	10.0	8	27.0
Scald burn	6	40.0	1	20.0	5	50.0	12	40.0
Electrical burn	2	13.3	0	0.0	3	30.0	5	16.5
Chemical burn	3	20.0	1	20.0	1	10.0	5	16.5
Total	**15**	**100.0**	**5**	**100.0**	**10**	**100.0**	**30**	**100.0**

*Only two isolates from two male patients were admitted to ICU due to RTA.

The thirty isolates of *B.cepacia* were revealed from the three department of burn units, half of them were isolated from male dep., followed by pediatric dep. and least were isolated from female dep. In both males and pediatric dep., Scald burn was the most cause of burn wound as *B.cepacia* infection was (40.0% and 50.0% respectively), compared to female department were flame was the main cause of burn associated with *B.cepacia* infection (60.0%). Table (7) and Figure (4)

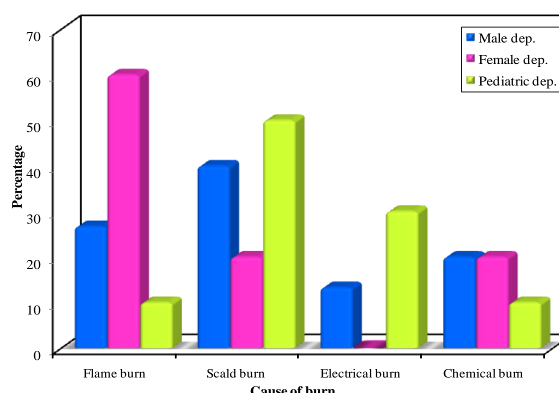

Figure (4): Frequency of *B.cepacia* isolates according to cause of burn.

Table (8): Susceptibility patterns of 32 BCC strains with various types of antibiotics

Susceptibility patterns tested antimicrobial	Susceptible (S)		Intermediate (I)		Resistant (R)		Total	
	No.	%	No.	%	No.	%	No.	%
piperacillin / Tazobactam (TZP)	0	0.0	0	0.0	32	100.0	32	100.0
Trimethropirn/ Sulfamethoxazole (SXT)	0	0.0	0	0.0	32	100.0	32	100.0
Ceftazidime (CAZ)	0	0.0	0	0.0	32	100.0	32	100.0
Chloramphenicol(C)	0	0.0	0	0.0	32	100.0	32	100.0
Tobramycin (TOB)	0	0.0	0	0.0	32	100.0	32	100.0
Imipinem (IPM)	11	34.0	5	16.0	16	50.0	32	100.0
Meropenem (MEM)	11	34.0	5	16.0	16	50.0	32	100.0
Tetracycline (TE)	13	40.5	4	12.5	15	47.0	32	100.0
Colistine	19	60.0	0	0.0	13	40.0	32	100.0

It is clear from Susceptibility patterns of 32 BCC strains with various types of antibiotics that *B.cepacia* isolates were 100% resistant to piperacillin / Tazobactam (TZP), Trimethropirn/ Sulfamethoxazole (SXT), Ceftazidime (CAZ), Chloramphenicol (C) and Tobramycin (TOB). Where 50% of isolates were resistant to Imipinem (IPM) and Meropenem (MEM), 47% to tetracycline (TE) and the least resistant was to Colistine (40%).

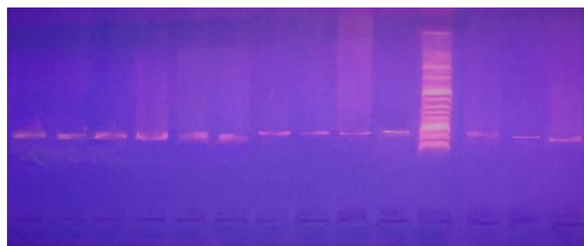

Figure (5): Multiplex PCR of BCC strains with positive recA gene at 1.040 bp.

Discussion

B. cepacia complex is now added to the list of nosocomial pathogens that cause serious problems in clinical settings because of its high transmissibility between hospitalized patients and its multiple drug resistance.[18]

The present study aimed to detect the isolation percentage of BCC from 115 patients who admitted to ICUs and burn units of Alexandria Main University Hospital during 6 months from April to October 2015. A total of 150 clinical samples were collected from these patients. The majority of clinical samples were swabs from burn wound 71(47.3%), followed by mini BAL specimen 31(20.7%), urine 29 (19.3%), blood 15(10%), tissue specimen 2 (1.3%), and only two specimen were CSF and pleural effusion (one of each) (Table: 5). Out of 150 clinical samples cultured, 128(85.3%) showed isolates and 22(14.7%) no growth. The majority of isolates causing burn wound infection were P.aeruginosa (31.0%), followed by *B.cepacia* (30.0%), E.coli (17.0%) and S.aureus (MRSA) (10.0%).(Table:4)

These results correspond to those reported by Omer N *et.al*,[19] found that out of 2079 specimen collected from patients in different wards of Alexandria Main University hospital during 6 months in 2011. A 150/2079 (7%) showed isolates of NFGNB were found to be oxidase positive from which 35/150 (23%) isolates were confirmed as BCCby Rap ID NF Plus. The highest rate of isolation of BCC was from burn wound 30/35 (85.7%) followed by sputum 4/35 (11.4%) and urine 1/35 (2.9%).

Other studies reported higher rates of isolation of BCC from specimens other than those in this study. Gales et al[3] found that out of 176 NFGNB collected from Latin America region through the surveillance program (1997-2002) 83 strains (83/176) belonged to *Burkholderia* spp.: 52/83 (62.7%) were from blood, 25/83 (30.1 %) were from sputum, 3 (3.6%) were from skin and soft tissue infection and 3 (3.6%) were from urine.

On the other hand, in a Turkish University Hospital, Dizbay et al in 2009[20]showed, that 39 strains of *B. cepacia* were isolated from various clinical specimens obtained from hospitalized patients mainly in the ICU and this constituted 1.0% of Gram negative isolates and was thus considered as a rare cause of nosocomial infection in this hospital. These study corresponded with our present study were BCC showed low rate in ICUs (1.8%). (Table: 4)

Generally, in the literature reports of nosocomial *B. cepacia* infections are usually restricted to nosocomial epidemics or outbreaks. Reports of sporadic cases of *B. cepacia* nosocomial infections are rare, probably due to the lack of specific laboratory tests in routine testing in most hospitals,

this *B. cepacia* has been ambiguously reported as NFGNB or simply *Pseudomonas* spp. This was the case in the routine microbiology laboratory before this study. This also explains the lack of reports about the prevalence of *B. cepacia* infections in Egypt and many countries.[21]

In Egypt, Ain Shams University Burn Unit,Nasser et al in 2003[21] reported that the most frequent isolate was *Pseudomonas aeruginosa* (21.6%), followed by *Klebsiella pneumoniae* (15.2%). Studying the time-related changes in burn wound microbial colonization showed an initial predominance of Gram-positive cocci upon admission (70.7%) over Gram-negative bacilli (27.6%). Burn wound sampling performed starting from the sixth day onwards, revealed further prevalence of Gram-negative bacilli (72.7%) over Gram-positive cocci (22.7%). Although different studies aimed at studying burn wound colonization and infections, it was noticed that BCC was not reported. Most probably all *B. cepacia* isolates were falsely regarded as *Pseudomonas* with no further identification. This is particularly justified in studies done before the actual transfer of BCC to the new genus *Burkholderia* in 1993.[1] However, several more recent studies have also failed to detect *B.* cepacia and maintained regarding all NFGNB oxidase positive as *Pseudomonas,* probably, because BCSA and other *Burkholderia* selecting media were not included in their routine isolation procedures.[2]

The use of the 3 criteria: motility, lysine and ornithine decarboxylation in the present study for the preliminary identification of *B. cepacia* as well as the elimination of other oxidase NFGNB organisms that grew on BCSA (*Ralstonia* spp. and *Pandoraea* spp.) was based on the findings reported by several other prior studies.[2]

In this study, out of 75 NFGNB oxidase positive isolates, 32/75 (42.6%) were preliminarily identified as BCC by motility, lysine and ornithine decarboxylases and were confirmed by ability to grow on BCSA. (Table: 4)

The choice of BCSA in this study was based on the findings of Henry et al in 1999[22] and Eram et al in 2004[18] who reported that BCSA was superior to other *B. cepacia* selective media, such as OFPBL and PCA agar in terms of rapidity and in suppressing oxidase positive organisms other than BCC. BCSA was able to support the growth of the BCC, *Ralstonia* spp. and *Pandoraea* spp., but inhibited significantly more non- BCC organisms than did OFPBL and PCA. This was also evidenced by Segonds et al in 2009[23] who found that all 18 respiratory isolates of *B. gladioli* from CF patients in his study grew on OFPBL and PCA, but only 13/18 (72%) grew on BCSA.

A study by Kiska et al in 1996[24] assessed the accuracy of several commercial systems for identification of *Burkholderia cepacia.* 150 non-fermenting bacilli, including 58 isolates of *B. cepacia* and other Gram-negative bacilli were tested. They found that the API 20NE system accurately identified 25/58(43%) of the *B. cepa*cia isolates, only 50% of the *B. cepacia* strains were correctly identified by using the Vitek system while the Rap ID NF Plus commercial system was found to be the most accurate commercial system for the identification of *B. cepacia*, this system correctly identified 50/58 (86%) *B. cepacia* strains.

Similarly, in a study in Alexandria Main University Hospital 2015, the most of BCC strains isolated were from surgical wards 27/35 (77.15%), followed by burn unit 24/35 (68.6%), medical wards 6/35 (17.15%) then low percentage from intensive care units 2/35 (5.7%). This may be attributed to significant thermal injuries that induce a state of immunosuppression, in burn patients, also the devitalized tissue and moist burn are favorable conditions for colonization of micro-organisms and subsequent infection.[19]

On the other hand, Dizbay et al(2009)[20] in Turkey found that most of BCC strains were isolated from the intensive care units 24/39 (61.5%) followed by medical wards 10/39 (25.6%) then surgical wards 5/39 (12.8%). In their study the ICUs were the wards in which nosocomial *B. cepacia* infections occurred more frequently (61.5%). The most frequent risk factors in these patients were invasive procedures such as mechanical ventilation and urinary and central

venous catheterization, which were mostly related to the severity of the underlying diseases of patients in the ICU.

In this study, *B.cepacia* isolates according to the results of antibiotic susceptibility testing by the disc diffusion method, were 100% resistant to piperacillin / Tazobactam (TZP), Ampicillin / sulbactam (SAM), Cefipime (FEP), Cefoperazone (CFP), Cefotaxime (CTX), Cefotriaxone (CRO), levofloxacin (LEV), Trimethropirn/ Sulfameth-oxazole (SXT), Ceftazidime (CAZ), Chloramph-enicol (C) and Tobramycin (TOB). Where 50% of isolates were resistant to Imipinem (IPM) and Meropenem (MEM), 47% to tetracycline and the least resistant was to Colistine (40%). (Table: 8)

These results correspond to those reported by Omer N *et.al*,[19] isolates ofBCC were most susceptible to meropenem (88.5%) followed by ceftazidime (60%), tobramycin (40%), chloramphenicol (37.1%), piperacillin/tazobactam (25.7%) and tetracycline (5.8%). While all strains (100%) were resistant to both co-trimoxazole and ciprofloxacin.

On the other hand, since their recognition in 1992, several studies tested BCC for antibiotic susceptibility. They all agreed that this organism was highly resistant to multiple antibiotics. Gautam et al in 2009[25] tested 30 strains of BCC isolated from septicemia patients in a tertiary care hospital in India, by the antibiotic disc diffusion method, they found that their isolates of BCC were susceptible to piperacillin–tazobactam (26/30, 86.7%), levofloxacin (25/30, 83.3%), ceftazidime (24/30, 80%) and tetracycline (23/30, 76.7%). And the least susceptibility was against meropenem (11/30, 36.7%) and co-trimoxazole (7/30, 23.3%).

Dizbay et al in 2009[20] tested 39 strains of nosocomial BCC isolated from cases of pneumonia, blood stream infections, surgical site and urinary tract infections in a Turkish University Hospital for antibiotic susceptibility. They reported that their *B. cepacia* isolates were most resistant to ceftazidime (24/39, 61.5%), followed by amikacin and ciprofloxacin (21/39, 53.8%) each, then meropenem (19/39, 48.7%), co-trimoxazole (22/39, 56.4%) and piperacillin–tazobactam (15/39, 38.4%).

Examining the results of those 3 studies, we observe that the strains were more sensitive to meropenem (50%) than those of Gautam et al[25] (36.7%) and similarly with Disbay et al (51.3%). The present study were more resistant to ceftazidime (100%) than those of Disbay et al (61.5%), yet the least resistant to ceftazidime was reported by Gautam et al (20%).[25]

More discrepancies are observed as regards piperacillin–tazobactam, where BCC strains in the study of Gautam et al[25] and Dizbay et al[20] were more sensitive to this antibiotic than those tested in the present study showing (86.7%, 61.6%) respectively, and the sensitivity for ciprofloxacin were (83.3%, 46.2%) while none of the 32 *B. cepacia* complex strains in the present study were sensitive to piperacillin–tazobactam and ciprofloxacin.

Another study in Alexandria University 2011,[19] the antibiotic resistance pattern of the 35 BCCstrains as detected by the modified Bauer Kirby single disc diffusion technique revealed that altogether the 35 strains were distributed among 17 different resistance profiles. The largest cluster of strains of identical resistance profile was from patients in the burn unit where 5 strains shared the resistance pattern (TE, SXT, CIP, TZP) and 4 strains shared the resistance pattern (TE, SXT, CIP, TZP, C) with resistance to chloramphenicol as the only added minor variation so the 9 strains could be safely accepted to have almost identical resistance pattern which may implicate a common source or origin. In addition, 3 strains shared the antibiotic resistance pattern (CAZ, TE, SXT, CIP, TOB, and C) and another 3 shared the pattern (TE, SXT, CIP, TOB, C, and TZP).

Conclusions

1) BCC and *P.aeruginosa* were the most predominant isolates caused burn infection.
2) The more the duration of stay in burn units and ICUs, the more infection rate.
3) The lowest percentage of BCC resistance was against meropenem and imipenem.

This finding requires further study of the environment in the burn unit in the near future. More strains of Bcc are to be isolated and typed from both the patients and the hospital environment to detect the source of cross contamination and infection.

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