Burkholderia Cepacia Complex Among Intensive Care Unit Patients In Two Private Hospitals In Alexandria

Hadir El Kady, Osama Nasr El-Deen Mohamed, Amani Farouk Abaza , Yasser Hassan Mousa Zidan

ABSTRACT: Burkholderia cepacia complex (BCC) is a group of 17 closely-related species that emerged as an important opportunistic pathogen. It causes serious infections in clinical settings because of its high intrinsic multiple antibiotic resistance and because it survives and multiplies in aqueous hospital environments including disinfectants. Infections caused by BCC include bacteremia, urinary tract infections, septic arthritis, peritonitis and respiratory tract infections; particularly in patients with cystic fibrosis (CF). Hemodialysis, permanence in intensive care units (ICUs), use of central venous catheters, and endotracheal tubes are recognized as risk factors contributing to BCC acquisition. B. cepacia selective agar (BCSA) is rapid efficient and for selective isolation of BCC. The RapID NF Plus is an accurate, rapid, easy system to identify BCC in four hours. This cross-sectional study aimed to study the occurrence of BCC infection among ICU patients in two private hospitals in Alexandria during a 4-month period from February to May 2016. Comparison of isolation of B. cepacia on conventional media and on BCSA, besides identification of isolates by RapID NF Plus system was also aimed at. Conventional media and BCSA medium were used to isolate and identify BCC in 150 examined samples. Further confirmation of BCC isolates was done using RapID NF Plus system and their antimicrobial susceptibility pattern was detected.Causative agents were isolated from 74% of samples. The infection rate was highest in the third week of hospitalization. BCC was isolated from 7.2% of samples. The highest rate of isolation occurred in patients with pulmonary diseases. All BCC isolates were found to be multiple drug resistant (MDR) but were highly susceptible to ceftazidime, meropenem, and piperacillin- tazobactam.

Key words: Burkholderia ; BCC; ICU patients; BCSA medium; RapID NF Plus system

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INTRODUCTION:

Burkholderia cepacia complex (BCC) is a group of 17 closely-related species that are ubiquitous in nature and found particularly in soil and water. For a long time, BCC was believed to be only a plant pathogen, but later on, it has emerged as an important opportunistic pathogen that causes serious infections in clinical settings, particularly among patients with prior broad-spectrum to antibiotic therapy [1]. Burkholderia cepacia (B. cepacia) formerly known as Pseudomonas cepacia, (P. cepacia) is an aerobic, motile, glucose non fermenting, multidrug resistant gram negative bacillus that proliferates under conditions of minimal nutrition and can survive in the presence of certain disinfectants. The most common species of BCC include: B. cepacia, B. multivorans and B.cenocepacia [2]. BCC has emerged as an important health care associated (HCA) pathogen causing morbidity and mortality in hospitalized patients, largely because of its high intrinsic antibiotic resistance. It is one of the most antimicrobial resistant organisms encountered. BCC is intrinsically resistant to antimicrobial agents such as aminoglycosides, first and second generation cephalosporins, antipseudomonal penicillins and polymyxins. In addition, it survives and multiplies in aqueous hospital environments, where it may persist for long periods; thus, giving an extreme value to its proper identification [3]. Infections caused by BCC include bacteremia, urinary tract infections, septic arthritis, peritonitis and respiratory tract infections particularly in patients with cystic fibrosis (CF). However, the pathogenicity of BCC is not always limited to CF or immunocompromised patients. Among non-CF hospitalized patients, hemodialysis, permanence in intensive care units (ICUs), use of central venous catheters, indwelling urinary catheters, and endotracheal tubes are recognized as risk factors contributing for BCC acquisition. Outbreaks of BCC septicemia have been documented worldwide in ICUs, oncology units and renal failure patients[4-7]. 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. These media include P. cepacia (PC) agar; oxidation fermentation polymyxin bacitracin lactose (OFPBL) agar, and B. cepacia selective agar (BCSA) [8-10]. A study by Shelly et al., identified BCC by different commercial systems and concluded that although misidentification is widespread; the RapID NF Plus system (an identification system based on enzyme technology to identify over 70 medically important, oxidase-positive, Gram-negative bacilli, including B. cepacia spp. in four hours) was accurate, rapid, easy to use and with relatively reproducible results when compared with

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those of the API 20NE and Vitek systems prior to supplemental testing [11]. With the poor laboratory proficiency prevailing worldwide; the accurate identification of BCC presents serious diagnostic difficulties to medical microbiology laboratories. Thus the use of appropriate selective media and identification procedures is vital for optimum culture and reliable diagnosis of BCC especially among critically ill patients [12].

MATERIALS AND METHODS:

Study design, sample size and study setting:

The present cross-sectional study was carried out during a 4-month period from February to May 2016. A total of 150 different clinical samples were collected from 118 patients who were admitted to the ICUs at Mabaret El Asafra hospital (Hospital A) with a capacity of 46 beds and Petroleum hospital (Hospital B) with a capacity of 20 beds in Alexandria. Selected patients presented with signs and symptoms of respiratory tract infections, urinary tract infections, skin and soft tissue infections (e.g. bed sores and diabetic ulcers), pyrexia of unknown origin or were on mechanical ventilators. A questionnaire sheet was completed for every patient enrolled in the present study and included all the relevant personal information and disease history.

Samples collection:

Thirty two mini-broncho-alveolar lavage (BAL), 31 sputum, 28 urine, 15 blood samples, 18 wound swabs, 10 pleural fluid, 9 ascitic fluid, 5 pericardial fluid and 2 CSF samples were aseptically collected on admission upon request of the treating physician by the assigned medical staff in the ICU according to the standard methods adopted for ICU patients.

Processing of samples:

For all samples the following was done according to standard methods of microbiological examination of samples: [13]

Macroscopic examination:

The appearance of each sample was observed to record its colour, aspect; if it is clear, purulent or mucopurulent or if it contains blood.

Microscopic examination:

Smears from each sample were Gram stained, then examined under oil immersion lens for the presence of white blood cells (WBCs), red blood cells (RBCs), squamous epithelial cells and any visible bacteria or fungi.

Culture:

Ten microns of the most viscous part of some samples or of the diluted sample in others, were streaked on the surface of blood agar, Mac Conkey agar and BCSA plates using a calibrated loop. Inoculated blood and Mac Conkey agar plates were incubated aerobically at 37°C for 24 hours. BCSA plates were incubated aerobically at 37°C for 48 to72 hours. Sputum samples were considered acceptable if ratio of PMN to squamous cells was more than 10:1. An equal volume of the sputum and sterile saline were mixed and then incubated at room temperature for 15 minutes with intermittent shaking for homogenization. The homogenized sputum was then diluted 1 to 100 in sterile saline before culture. Urine samples were subjected to direct microscopic examination besides Gram stain. Two loopfuls of well mixed fresh uncentrifuged urine were examined for the presence of pus cells, squamous epithelial cells, RBCs, casts, crystals and the presence of bacteria. The significant pyuria was defined as > 8-10 PMN/mm3. Blood culture bottles were incubated aerobically over night at 37°C. The bottles were inspected daily for hemolysis of RBCs or turbidity in the liquid portion, or blindly after 48 hours subculture of 1 ml of aspirated broth culture was done. The blood culture bottles were examined daily for 7 days and were considered negative and discarded if no signs of growth were detected. Suspension of wound swab in sterile peptone water, besides, pleural, ascetic, pericardial fluids and CSF samples were centrifuged at 2000 rpm for 15 min and sediment was collected and used for direct smear and culture.

Identification of BCC isolates: [1,13]

Cultural characters:

All isolates that appeared as smooth and slightly raised, occasionally mucoid, non-hemolytic on blood agar plates, non-lactose fermenting on Mac Conkey agar plates and that varied from grey to sage green with the medium changing from orange to bright pink on BCSA plates were suspected as BCC isolates.

Biochemical reactions:

Isolates that appeared as non-capsulated, non sporulating Gram negative bacilli, with no special arrangement and were motile, catalase positive, oxidase positive, arginine dihydrolase negative, lysine decarboxylase positive, ornithine decarboxylase positive, indole negative, methyl red negative, Voges-Proskauer negative, citrate positive, and showed alkaline slant/no change on the butt with no H2S or gas on TSI were considered as BCC species. They were further identified using:

RapID NF Plus system: [14,15]

Procedure: All suspected strains were subcultured on Mac Conkey's agar. Well isolated colonies of the same morphological type of the organism were suspended in RapID inoculation fluid (1ml) to achieve a visual turbidity equal to 0.5 McFarland turbidity standard. Suspensions were used within 15 minutes of preparation for inoculation of RapID NF Plus Panels according to the instructions of the manufacturer. Inoculated panels were incubated at 35-37°C in the incubator for 4 hours.

Scoring of RapID NF Plus panels:

Seven of the cavities were bifunctional, containing two separate tests in the same cavity. Results were first scored before the addition of reagent providing the first test result and then the same cavity was scored again after the addition of reagent to provide the second test result. Two drops of RapID NF Plus reagent were added from the cavities 4 through 8 , 2 drops of RapID Spot indole reagent were added to cavity 9 (URE/IND) and 2 drops of RapID Nitrate reagent were added to cavity 10 (GLU/NO3). Thirty seconds to 3 minutes were allowed for colour development

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then cavities 4 through 10 were scored again. Results were interpreted using Electronic RapID Compendium (ERIC) for identification by the microcode obtained from panels.

Antibiotic susceptibility testing

The antibiotic sensitivity pattern of the eight identified BCC isolates was detected using the disc agar diffusion procedure on Muller Hinton agar: Modified Kirby-Bauer antibiotic sensitivity test (Bauer et al.,1966) [16]. The inhibition zone diameters were measured and interpreted as recommended by the Clinical and Laboratory Standards Institute (CLSI) [17].

Statistical analysis: [18]

The required samples were selected randomly using simple random technique. Results were statistically analyzed using Package for Social Sciences (SPSS /version 20.0) software. The significance level (0.05 parametric) was used to indicate statistical significance.

RESULTS:

In the current study, 80/118 (67.8%) of the studied patients, were admitted to the ICU of hospital A while 38/118 (32.2%) were admitted to the ICU of hospital B. Males represented 64.4% of the study sample while 35.6% of which were females. In both hospitals, the highest percentage of patients were of age group >60-80 years (30% and 31.6%, respectively). The mean age value of males and females in hospital A were 49.83 and 47.39 years, respectively. The majority of patients were admitted to ICU suffering from pulmonary diseases and road traffic accidents [RTA] (17.8%) each, followed by cerebrovascular stroke [CVS] (16.1%), renal failure (10.2%), heart diseases (8.5%), hepatic diseases (6.8%), burn injury (5.9%), Leukemia and cancer and acute pancreatitis (5.1%) each. On the other hand, postoperative period and disseminated intravascular coagulation (DIC) cases were the minority of cases (4.2% and 2.5% respectively). In the current work, 150 samples were examined: 98 were from hospital A; 73 of which yielded microbial growth and 52 were from hospital B ; 38 of which were positive on culture. Thus, causative agents were isolated and identified in 111/150 samples (73%). (Table 1).

Table 1. *Distribution of the 150 collected samples in hospitals A and B according to type of samples and culture results.*

Type of samples	Hospital A						Hospital B						Total (n = 150)	
	Culture result				**Total (n =98)**		**Culture result**				**Total (n = 52)**			
	Growth (n = 73)		**No growth (n = 25)**				**Growth (n = 38)**		**No growth (n = 14)**					
	N	%	N	%	N	%	N	%	N	%	N	%	N	%
Mini-BAL	14	70.0	6	30.0	20	20.4	10	83.3	2	16.7	12	23.1	32	21.3
Sputum	17	77.3	5	22.7	22	22.4	8	88.9	1	11.1	9	17.3	31	20.7
Urine	17	89.5	2	10.5	19	19.4	10	90.9	1	9.1	11	21.2	30	20.0
Wound swabs	10	76.9	3	23.1	13	13.3	4	100.0	0	0.0	4	7.7	17	11.3
Blood	5	45.5	6	54.5	11	11.2	1	25.0	3	75.0	4	7.7	15	10.0
Pleural fluid	5	100.0	0	0.0	5	5.1	4	80.0	1	20.0	5	9.6	10	6.7
Ascitic fluid	2	50.0	2	50.0	4	4.1	1	25.0	3	75.0	4	7.7	8	5.3
Pericardial fluid	3	100.0	0	0.0	3	3.1	0	0.0	2	100.0	2	3.8	5	3.3
CSF	0	0.0	1	100.0	1	1.0	0	0.0	1	100.0	1	1.9	2	1.3
Total	73	74.5	25	25.5	98	100.0	38	73.1	14	26.9	52	100.0	150	100.0
□□□MCp□	12.674(0.086)						18.783*(0.004*)							

χ2, p: χ2 and p values for Chi square test for comparing between the two groups.
MC: Monte Carlo for Chi square test.
*: Statistically significant at p ≤ 0.05.

It is clear that when the duration of stay in ICU increased, the infection rate increased ranging from 28.6% in the first week up to 88.9% in the third week in hospital A, and from 14.3% in the first week to 90.9% in the third week in hospital B. This relation was found to be statistically significant (p<0.002). (Table 2)

Table 2. *Relation between culture results and duration of stay in ICU in hospitals A and B.*

Duration of stay	**Hospital A**						**Hospital B**						**Total (n = 150)**	
	Culture result				**Total (n = 98)**		**Culture result**				**Total (n = 52)**			
	Growth (n = 73)		**No growth (n = 25)**				**Growth (n = 38)**		**No growth (n = 14)**					
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
First week	4	28.6	10	71.4	14	14.3	1	14.3	6	85.7	7	13.5	21	14.0
Second week	53	80.3	13	19.7	66	67.3	27	79.4	7	20.6	34	65.4	100	66.7
Third Week	16	88.9	2	11.1	18	18.4	10	90.9	1	9.1	11	21.2	29	19.3
Total	73	74.5	25	25.5	98	100.0	38	73.1	14	26.9	52	100.0	150	100.0
MCp	15.868*(<0.001*)						12.376*(0.002*)							

χ2, p: χ2 and p values for Chi square test for comparing between the two groups.
MC: Monte Carlo for Chi square test.
*: Statistically significant at p ≤ 0.05.

Gram negative bacilli were isolated from 81/111 (73%) of examined samples, Gram positive cocci were isolated from 24/111 (21.6%) of samples, while only 6/111 (5.4%) of the examined samples yielded fungi. (Table 3)

Table 3. *Distribution of 111 isolates from the studied samples in hospitals A and B.*

Isolated organisms	**Frequency of isolation**					
	Hospital A (n = 73)		**Hospital B (n = 38)**		**Total (n = 111)**	
	No.	**%**	**No.**	**%**	**No.**	**%**
Gram negative bacilli:	52	71.2	29	76.3	81	73.0
Escherichia coli	10	13.7	10	26.3	20	18.0
P. aeruginosa	13	17.8	5	13.2	18	16.2
Klebsiella pneumoniae	12	16.4	5	13.2	17	15.3
Proteus mirabilis	3	4.1	1	2.6	4	3.6
Acinetobacter baumannii complex	8	11.0	4	10.5	12	10.8
Burkholderia cepacia complex	6	8.2	2	5.3	8	7.2
Stenotrophomonas maltophilia	0	0.0	2	5.3	2	1.8
Gram positive cocci:	17	23.3	7	18.4	24	21.6
Staphylococcus aureus	11	15.1	5	13.2	16	14.4

Streptococcus pneumoniae	3	4.1	2	5.3	5	4.5
Enterococcus faecium	3	4.1	0	0.0	3	2.7
Fungi :	4	5.5	2	5.3	6	5.4
Candida albicans	4	5.5	2	5.3	6	5.4
χ^2 (MCp)	7.739(0.674)					

$\chi2$, p: $\chi2$ and p values for Chi square test for comparing between the two groups.
MC: Monte Carlo for Chi square test.

BCC represented 8/111 (7.2%) of the isolates; six from hospital A and two from hospital B. The highest rate of BCC infection occurred among males (75%) and among those aged 60-80 years (50%). Maximum isolation of BCC occurred in the third week of stay in the ICU (62.5%) , among patients with pulmonary diseases (37.5%) and was from sputum samples (50%). The highest rate of isolation of BCC occurred in February (50%), followed by March (37.5%), and the lowest rate of isolates was recorded in April (12.5%). The difference between these figures was not statistically significant (p= 1.000). Tables (4 and 5) illustrate the frequency of isolation of different isolates from both hospitals. It is evident that P. aeruginosa was the most frequently isolated agent in hospital A : 13/73 (17.8%), while in hospital B E.coli showed the highest frequency of isolation among isolates: 10/38 (26.3%).

Table 4. *Distribution of isolates according to type of sample in hospital A.*

Isolated organisms	Type of samples																Total (n = 73)	
	Mini-BAL (n = 14)		Sputum (n = 17)		Urine (n = 17)		Wound (n = 10)		Blood (n = 5)		Pleural fluid (n = 5)		Ascitic fluid (n = 2)		Pericardial fluid (n = 3)			
	No.	%	No	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
E.coli	0	0.0	1	5.9	7	41.2	1	10.0	0	0.0	0	0.0	1	50.0	0	0.0	10	13.7
P. aeruginosa	3	21.4	1	5.9	4	23.5	4	40.0	0	0.0	0	0.0	1	50.0	0	0.0	13	17.8
K. pneumoniae	3	21.4	6	35.3	1	5.9	0	0.0	1	20.0	1	20.0	0	0.0	0	0.0	12	16.4
P. mirabilis	0	0.0	0	0.0	3	17.6	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	3	4.1
Acinetobacter baumannii complex	3	21.4	1	5.9	0	0.0	2	20.0	2	40.0	0	0.0	0	0.0	0	0.0	8	11.0
BCC	0	0.0	4	23.5	1	5.9	0	0.0	1	20.0	0	0.0	0	0.0	0	0.0	6	8.2
Stenotrophomonas maltophilia	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
S. aureus	2	14.3	2	11.8	0	0.0	2	20.0	1	20.0	3	60.0	0	0.0	1	33.3	11	15.1
S. pneumoniae	1	7.1	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	2	66.7	3	4.1
E. faecium	1	7.1	0	0.0	0	0.0	1	10.0	0	0.0	1	20.0	0	0.0	0	0.0	3	4.1
C. albicans	1	7.1	2	11.8	1	5.9	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	4	5.5
Total	14	100.0	17	100.0	17	100.0	10	100.0	5	100.0	5	100.0	2	100.0	3	100.0	73	100.0
χ^2 (MCp)	77.405*(<0.001*)																	

$\chi2$, p: $\chi2$ and p values for Chi square test for comparing between the two groups.
MC: Monte Carlo for Chi square test.
*: Statistically significant at p ≤ 0.05.

Table 5. *Distribution of isolates according to type of sample in hospital B.*

Isolated organisms	Type of samples																Total (n =38)	
	BAL (n = 10)		Sputum (n = 8)		Urine (n = 10)		Wound (n = 4)		Blood (n = 1)		Pleural fluid (n = 4)		Ascitic fluid (n = 1)		Pericardial fluid (n = 0)			
	No.	%	No	%	No.	%	No.	%	No	%	No.	%	No	%	No.	%	No.	%
E.coli	4	40.0	0	0.0	6	60.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	10	26.3
P. aeruginosa	0	0.0	0	0.0	1	10.0	2	50.0	0	0.0	2	50.0	0	0.0	0	0.0	5	13.2
K. pneumonia	2	20.0	2	25.0	1	10.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	5	13.2
P. mirabilis	0	0.0	0	0.0	1	10.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	2.6
Acinetobacter baumannii complex	2	20.0	1	12.5	0	0.0	0	0.0	1	100.0	0	0.0	0	0.0	0	0.0	4	10.5
BCC	0	0.0	0	0.0	1	10.0	1	25.0	0	0.0	0	0.0	0	0.0	0	0.0	2	5.3
Stenotrophomonas maltophilia	0	0.0	2	25.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
S. aureus	0	0.0	1	12.5	0	0.0	1	25.0	0	0.0	2	50.0	1	100.0	0	0.0	5	13.2
S. pneumonia	1	10.0	1	12.5	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	2	5.3
E. faecium	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	2	5.3
C. albicans	1	10.0	1	12.5	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	2	5.3
Total	10	100.0	8	100.0	10	100.0	4	100.0	1	100.0	4	100.0	1	100.0	0	0.0	38	100.0
□□□MCp□	68.876*(0.004*)																	

χ^2, p: χ^2 and p values for Chi square test for comparing between the two groups.
MC: Monte Carlo for Chi square test. *: Statistically significant at $p \le 0.05$.

BCC isolates were 100% susceptible to ceftazidime, meropenem, and piperacillin tazobactam and were all MDR. (Table 6)

Table 6. *Antibiotic susceptibility test of the eight isolated BCC.*

Isolated organisms	Ceftazidime	Meropenem	Piperacillin-Tazobactam	Cefepime	Trimethoprim-sulfamethoxazole	Minocycline	Colistin	Levofloxacin	Ticarcillin-clavulante	Ciprofloxacin	Amikacin	Ampicillin	Ampicillin-sulbactam	Cefadroxil	Cefoprazone	Cefoprazone-sulbactam	Cefotaxime	Cefoxitin	Ceftriaxone	Cefuroxime sodium	Doxycyclin	Ertapenem	Gentamicin	Imipenem	Line Zolid	Moxifloxacin	Nalidixic Acid	Nitrofurantoin	Norfloxacin	Ofloxacin	Rifampicin	Teicoplanin	Tobramycin	Vancomycin
Hospital A																																		
	√	√	√	√	√	√	√	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×
	√	√	√	√	√	√	√	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×
	√	√	√	√	√	√	√	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×
	√	√	√	√	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×
	√	√	√	√	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×
	√	√	√	×	√	√	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×
Hospital B																																		
	√	√	√	√	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×
	√	√	√	√	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×

√=Sensitive.
×=Resistant.

DISCUSSION:

There is growing evidence that BCC is highly virulent in patients with CF and in patients with CGD. The most problematic characteristic of BCC is its high transmissibility between hospitalized patients from one patient to another, both within and outside the hospital and its multiple drug resistance [19]. BCC infection may ensue a significant illness, particularly in the ICU, where patients are at greater risk of nosocomial infection due to their underlying illness, proximity and need for invasive devices and procedures [5]. 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 poor laboratory proficiency in identification of this organism in routine testing in most hospitals [20]. B.cepacia has been ambiguously reported as NFGNB or simply as Pseudomonas spp. This also explains the lack of reports about the prevalence of B. cepacia infections in Egypt and many countries [13,21]. The present study aimed to detect the isolation rate of BCC from patients in the ICU of Mabaret El Asafra hospital (A) and Petroleum hospital (B) according to the site of infection (specimen) throughout a period of 4 months (February to May 2016). During this period, 150 clinical specimens were collected from 118

patients who were admitted to the ICUs of both hospitals. BCC was isolated and confirmed by RapID NF Plus from 8/150 (5.3%) of cultured specimens. The highest rate of isolation of BCC was from sputum 4/8 (50%) followed by urine 2/8 (25%), wound and blood samples 1/8 (12.5%), each. Other studies reported higher rates of isolation of BCC than those in our study. Bhise et al., (2013) isolated BCC out of 100 % of cases of neonatal septicemia in the ICU [12]. Gales et al., found that 47% of the NFGNB strains collected from Latin America region through the surveillance program (1997-2002) belonged to Burkholderia species: 62.7% of which were isolated from blood, 30.1% from sputum, 3.6% from skin and soft tissue infection and 3.6% from urine [22]. On the other hand, a lower BCC infection rate of 1.7 % was reported by Omar et al., (2015). The highest percentage of which was isolated from pus (85.7%) followed by sputum (11.4%) and urine (2.9%) [23]. In a Turkish University Hospital, (2009) most of BCC strains were isolated by Dizbay et al., from the ICUs (61.5%). The most frequent risk factors in these patients were invasive procedures such as mechanical ventilation, urinary and central venous catheterization, which were mostly related to the severity of the underlying diseases of such patients [24]. In the current study, 20 out of 111 isolates (18%) were able to grow on BCSA; 8/20 (40%) were preliminarily identified as BCC by oxidase test, motility, lysine and ornithine decarboxylases and then confirmed by RapID NF Plus. The choice of BCSA in this study was based on the findings of Henry et al., (1999) [25], Eram et al., (2004) [26] and Omar et al., (2015) [23] who reported that BCSA was superior to other B. cepacia selective media, such as OFPBL and PCA in terms of rapidity and in suppressing oxidase positive organisms other than BCC. This was also evidenced by Dizbay et al., (2009) who found that all his respiratory isolates of B. gladioli from CF patients grew on OFPBL and PCA, but only 72% of which grew on BCSA [24]. A study by Kiska et al., (1996) assessed the accuracy of several commercial systems for identification of B.cepacia. RapID NF Plus commercial system correctly identified 86% of B. cepacia strains while only 43% and 50% of which were identified using API 20NE system and the Vitek system, respectively [27]. In 2000 Shelly et al., further studied several hundred clinical BCC isolates from different laboratories. They identified BCC by different commercial systems and concluded that although misidentification is widespread; the RapID NF Plus system was accurate, truly rapid, easy to use and that the results were easy to interpret and relatively reproducible when compared with those of the API 20NE and Vitek systems. Because the RapID NF Plus system is enzyme based and uses carbon substrate assimilation, its ability to identify weakly oxidizing B. cepacia isolates and atypical P. aeruginosa isolates may be enhanced compared with those of the other commercial systems [11]. In the present study, we therefore relied solely on the RapID NF Plus system for the identification of the 8 BCC isolates. The identity of all strains (100%) was confirmed biochemically by this system to be B. cepacia further proving that it is the most reliable test. BCC is intrinsically resistant to antimicrobial agents such as aminoglycosides, first and second generation cephalosporins antipseudomonal penicillins and polymyxins. As per the CLSI 2010 guidelines, the drugs recommended against BCC are ceftazidime, minocycline, meropenem and cotrimoxazole [28]. In the current work, according to the results of antibiotic susceptibility testing by the disc diffusion method, the 8 BCC isolates were most susceptible to ceftazidime, meropenem and piperacillin-tazobactam (100%) each, followed by cefepime (87.5%), co-trimoxazole and minocycline (50%) each and colistin (37.5%). All strains (100%) were resistant to both ciprofloxacin and ticarcillin-clavulante. In agreement with the current findings, Paul et al., (2016) reported the highest susceptibility of their BCC isolates to ceftazidime and their maximum resistance to ciprofloxacin[10]. Omar et al., (2013) also reported the highest sensitivity to both ceftazidime and meropenem and a 100% resistance to ciprofloxacin [23]. Besides, katsiari et al., (2012) also reported 100% susceptibility of their isolates to both meropenem and piperacillin/tazobactam but unlikely only limited susceptibility to ceftazidime (30%) [29]. Comparing the results of our study to those reported by Gautam et al., (2009) [30] and Dizbay et al., (2009) [24], we observed that our strains were much more sensitive to ceftazidime, meropenem and piperacillin–tazobactam. On the other hand, none of our 8 BCC isolates showed sensitivity to ciprofloxacin, while 83.3% of Gautam et al., strains and 46.2% of Dizbay et al., strains were sensitive. Variations of antibiotic susceptibility results are probably explained by the different antibiotic policies used in different countries. Such findings emphasize the need to isolate and test reliably more strains of BCC to review the therapeutic measures (that rely mainly on the use of proper antibiotics).

CONCLUSION:

Introduction of BCSA medium for screening of BCC strains supports the growth of Burkholderia, C. albicans, K. pneumoniae, and E. coli but totally inhibits Pseudomonas Spp. RapID NF Plus system is accurate in confirming the identity of BCC. Ceftazidime, meropenem and piperacillin-tazobactam followed by cefepime are good therapeutic options for BCC.

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