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Antibiotics Resistance among Nosocomial *Burkholderia cepacia* Isolates Detected in Sebha, Libya

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ABSTRACT

Background: *B. cepacia* complex (Bcc) is an emerging pathogenic organism that can cause many nosocomial infections among hospitalized patients. Inadequate laboratory facilities for *B. cepacia* complex detection and subsequently inappropriate treatment are considered a major cause for poor therapy outcomes.

Methods: This project was aimed to investigate phenotype production of ESBL, AmpC, and Carbapenemase among 47 *B. cepacia* complex isolated from different Sebha health care facilities.

Results: Our data showed that 44.68% were ESBL producers, 57.44% were AmpC producers, while only 29.78% produced carbapenemase. In this study, antibiotics susceptibility of Bcc isolates was variable, 100 % resistant to Ticarcillin/clavulanic acid, 85 % resistant to sulfamethoxazole-trimethoprim, 76 % resistant to Ticarcillin/clavulanic Chloramphenicol, 57 % to Ceftazidime, and 55 % to Tetracyclines, 44% to Ciprofloxacin and 31% to Meropenem.

Conclusion: In conclusion, this study shows that Bcc species have a higher resistance level attributed to several mechanisms. This high resistance needs careful antimicrobial prescribing regulations, and urgent implementation of infection prevention control is necessary.

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Introduction

Burkholderia cepacia complex (Bcc) is a group of emerging nosocomial gram-negative species with high intrinsic resistance to the most available clinical antimicrobial agents (1). Its an increasingly recognized cause of various nosocomial diseases, including respiratory infection, especially in cystic fibrosis (CF) patients, urinary tract, and septic infections (2). Furthermore, this organism is resistant to a broad spectrum of antibiotics, making treatment of these infections difficult and potentially increasing the morbidity and mortality rates (3, 4).

In North Africa, there is increasing concern about antimicrobial resistance, and it has been reported that 90% of Gram-negative bacteria are resistant to many antibiotics (5). *Burkholderia* cepacia has become a significant problem in health care centers and can be transmitted through the environment or nosocomially through exposure to infected drugs and equipment and person-to-person contact (6).

Currently, the resistance of *Burkholderia* cepacia species to antibiotics has intensively been reported. Several studies have reported increased antibiotic resistance (e.g., Meropenem, Ceftazidime) (7). It has been documented that Bcc resistance to carboxypenicillins, first and second-generation cephalosporins, correlate with the production of inducible ambler class A β-lactamases (8, 9). Also, resistance to a broad spectrum of b-lactam antibiotics, including piperacillin, Ceftazidime, and aztreonam. Although ESBL was chromosomally encoded, it is not a feature of the Bcc species (10). Other reports increased resistance due to serine carbapenemase production (11), another report that Bcc produce a diverse class of AmpC β-lactamase (12), which plays a role in cephalosporins resistance.

However, the lack of reports concerning the antibiotic susceptibility of Bcc in North Africa might be probably due to the lack of specific laboratory tests in the routine investigation in most laboratories in Africa. Moreover, Bcc phenotypic resistance mechanisms are not well studied. Also, limited access to genotypic methods rendered the detection of β-lactamases very difficult.

This study was carried out to study the antibiotic susceptibility pattern and to use phenotypic detection methods to detect the ESBL, Ampc β-lactamases, and carbapenemase mechanism generated by nosocomial Bcc isolates from health care facilities

Materials and Methods

Study strains and sample collection

Forty-seven strains were used in the study provided by (research laboratory faculty of science). The strains were collected from patients (pus, urine, sputum) in different health care facilities in Sebha, South of Libya.

Isoaltes identification

47 Bcc species were identified Using USP chapter <60> guidelines (14).phenotypic biochemical characteristics were done according to (13), (15), the confirmation of isolates was carried out according to the API20NE (bioMerieux) identification manual.

Antimicrobial Susceptibility testing

Antimicrobial susceptibility testing was performed by Kirby-Bauer protocol (disk diffusion) method according to Clinical and Laboratory Standards Institute (CLSI) (99) recommendations by using Muller Hinton Agar MHA. The following antimicrobial disks (Bioanalyse Co. ITALY) were used Ticarcillin/Clavulanic Acid TIM (85µg), Ceftazidime CAZ (30µg), Meropenem MEM (10µg), Tetracycline TE (10µg), Chloramphenicol C (30µg), Ciprofloxacin CIP (5µg), (Sulfamethoxazole / Trimethoprim) SXT (25ug).

McFarland 0.5 turbidity standard was used in this experiment. Plates were incubated at 37°C±2 for 16 to 18 hours. The diameter of the inhibition zone was measured in millimeters, and the result was interpreted regarding the (CLSI) (16).

Phenotypic detection of ESBLs production

Phenotypic detection of ESBL was done by a modified double-disc synergy test (DDST). Fresh colonies of tested bacteria were inoculated in sterile water, adjusted to McFarland 0.5, and then streaked on an MHA plate. A disc of amoxicillin-clavulanate (20/10 µg) was placed on the Muller Hinton agar (MHA) plates. Discs of cefotaxime (30 µg) and Ceftazidime (30 µg) were kept 20 mm apart from the amoxicillin-clavulanate disc. The plates were incubated aerobically at 37°C overnight. The enhancement of the inhibition zone around cephalosporin discs towards amoxicillin-clavulanate disc was taken as evidence of ESBL production. (keyhole phenomena).

Screening for AmpC β-lactamase production

The resistance to cefoxitin 30µg was used to screen for AmpC β-lactamase producers Bcc isolates. Based on the CLSI (16) criteria, all isolates showing an inhibition zone of <18 mm were considered as AmpC β-lactamase producers and subjected to a confirmatory test using two disk cefoxitin combined with 120ug boric acid and Tris-EDTA ethylenediaminetetraacetate (EDTA) with boric acid.

Carbapenemase-production test

Reference strain (*E. coli*) was used in this experiment, 0.5 McFarland (*E. coli*) adjusted suspension tube Inoculated on MHA plate, then a carbapenem disc (meropenem 10ug) was placed at the center of the plate. The test strains of bcc were Streaked as 3-5 from the center to the periphery of the plates and were then Incubated at 37 ºC for 18-24h. The presence of a distorted inhibition zone due to the growth of the indicator strain toward the meropenem disc is interpreted as a positive result..

Statical analysis

For statistical analysis, Minitab version 19 (Minitab LLC) software was used for the study of variance (ANOVA), two-sample t-test; in all cases, a P-value was considered indicative of significance if it was equal to or less than 0.05.

Results

The susceptibility patterns of 47 BCC isolates to antibiotics were performed using the disc diffusion method (Table 1). In this study, Bcc isolates were 100 % resistant to Ticarcillin/clavulanic acid, 85 % resistant to sulfamethoxazole-trimethoprim, 76 % resistant to Chloramphenicol, 57 % and 55 % to Ceftazidime and Tetracyclines respectively. On the other hand, the resistance of BCC isolates to Ciprofloxacin and Meropenem were 44% and 31%, respectively; the highest rate of resistance was observed in the carboxypenicillin group with 100% resistance and 85% to sulfonamides, lower resistance was detected in fluoroquinolones (45%) and to carbapenems (32%). (Fig 1).

Regarding ESBL production, it was only positive in 26 Bcc isolates (44.68%), where the inhibition zone toward Ceftaxim and Ceftriaxone was more than 5mm larger, and no production was observed in remaining (21/47) (55.32%) isolates (Fig 2A).

AmpC β-lactamases production was observed in 57.44% (27/47) while 44.68% (20/47) were negative (Fig. 25). The inhibition zone to cefoxitin (CX) with boric acid (BA) and EDTA increased compared to cefoxitin alone which indicated the presence of AmpC β-lactamases (Fig2B). Only 29.78 % (14/47) were positive for carbapenemase production by Modified Hodge test and showed a

resistance zone for meropenem 10µg (Fig 2C). The remaining 70.21 % (33/47)were negative for carbapenemase production.

Discussion

The *B. cepacia* complex species is a multidrug resistance pathogen and is considered a significant pathogen that mainly affects cystic fibrosis patients and endangers their lives (17). The infections caused by Bcc are usually treated with broad-spectrum antibiotics. The intrinsic resistance of these organisms to broad-spectrum antibiotics made the treatment of infected patients more difficult. It has been noticed that the antibiotic resistance of these Gram-negative bacteria has significantly been increased during the last few decades, and the therapeutic efficacy of many applied antibiotics is reduced by their expression of several β-lactamases enzymes (18). In addition, the pathogenicity of these bacteria is promoted by several virulence factors, including biofilm, siderophore production, lipase enzyme, and capsule formation (19). Resistance to drying and many disinfectants allows maintenance of Bcc on environmental surfaces (20) and help the transmission of these pathogens between the hosts (21). Further, It has been reported that *Burkholderia* species can develop resistance mechanisms under antibiotic pressure (22). However, the problem has become more complicated by the development of cross-resistance between different classes of antibiotics (22).

The use of trimethoprim-sulfamethoxazole is still used to treat chronic *B. cepacia* complex infections. However, it showed poor activity against many *B. cepacia* complex strains, as reported (23). In this study, we also observed that the isolated strains were 85 % resistant to trimethoprim-sulfamethoxazole, Which may indicate that it is not a drug of choice to treat this kind of infection. This finding was similar to the result obtained by other researchers (23). Our data has also shown that Bcc isolates were 100% resistant to Ticarcillin/clavulanic, consistent with previous studies (24). The Resistance of Bcc to β-lactam agents is mainly mediated by constitutively expressed or inducible chromosomal β lactamases or efflux pumps (25).

Carbapenems are highly effective against gram-negative pathogens. Emerging resistance to carbapenem, including imipenem and Meropenem, has also been reported, especially in cystic fibrosis patients infected with Bcc (23). The Carbapenem resistance is mainly mediated via efflux pumps (25). Our data showed that the resistance to Meropenem was 31% which is the lowest resistance rate among all applied antibiotics in this study. This may suggest that carbapenem can still be used to treat BCC infection and should therefore remain drugs of last resort. This result is supported by previous reports showing that Meropenem can be an alternative for Bcc infection, mainly when other antibiotics are ineffective (26). In this study, the resistance to Ciprofloxacin was observed, where 44% of the collection was resistant. Our finding endorses the result of studies showing that the Bcc has become more resistant if it was grown as biofilm (25), (27, 28, 29). Our study showed that the resistance to Chloramphenicol is high (76%). This finding is in agreement with other results showed by other authors (25).

Moreover, the resistance of Bcc to β-lactam, Ceftazidime has been recorded, Although this antibiotic was the first line for treating Bcc infections for many years which may increase the mortality rate if the therapy not switched to another drug at the proper time (30, 31), In our study, we found that the resistance to CAZ was 57% which confirms other reports regarding this point (31, 32, 33).

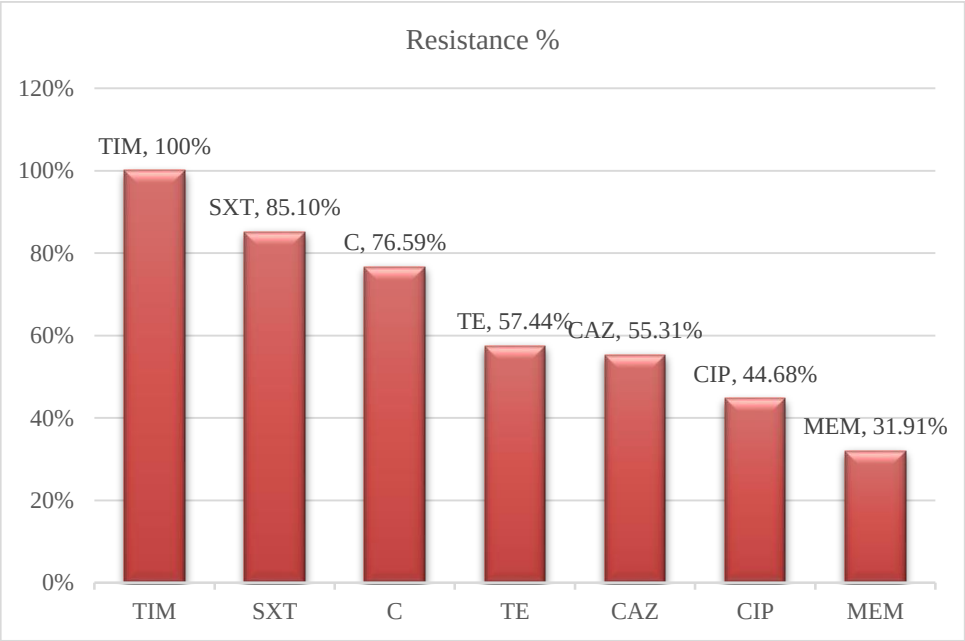

Fig 1. Antimicrobial resistance profile of Bcc isolates studied in this project.

Table 1. Antimicrobial resistance (AMR) profile among Bcc collection.

Group	Antibiotic	Resistance strains	Significance of AR" between Groups	
		No of Strains	%	P<0.5
Sulfonamides	sulfamethoxazole-trimethoprim	40	85	
Fluoroquinolones	Ciprofloxacin	21	45	
Cephalosporines	Ceftazidime	26	55	
Carboxypenicillin	Ticarcillin/clavulanic acid	47	100	
Chloramphenicol	Chloramphenicol	36	77	
Tetracyclines	Tetracyclines	27	57	
Carbapenems	Meropenem	15	32	

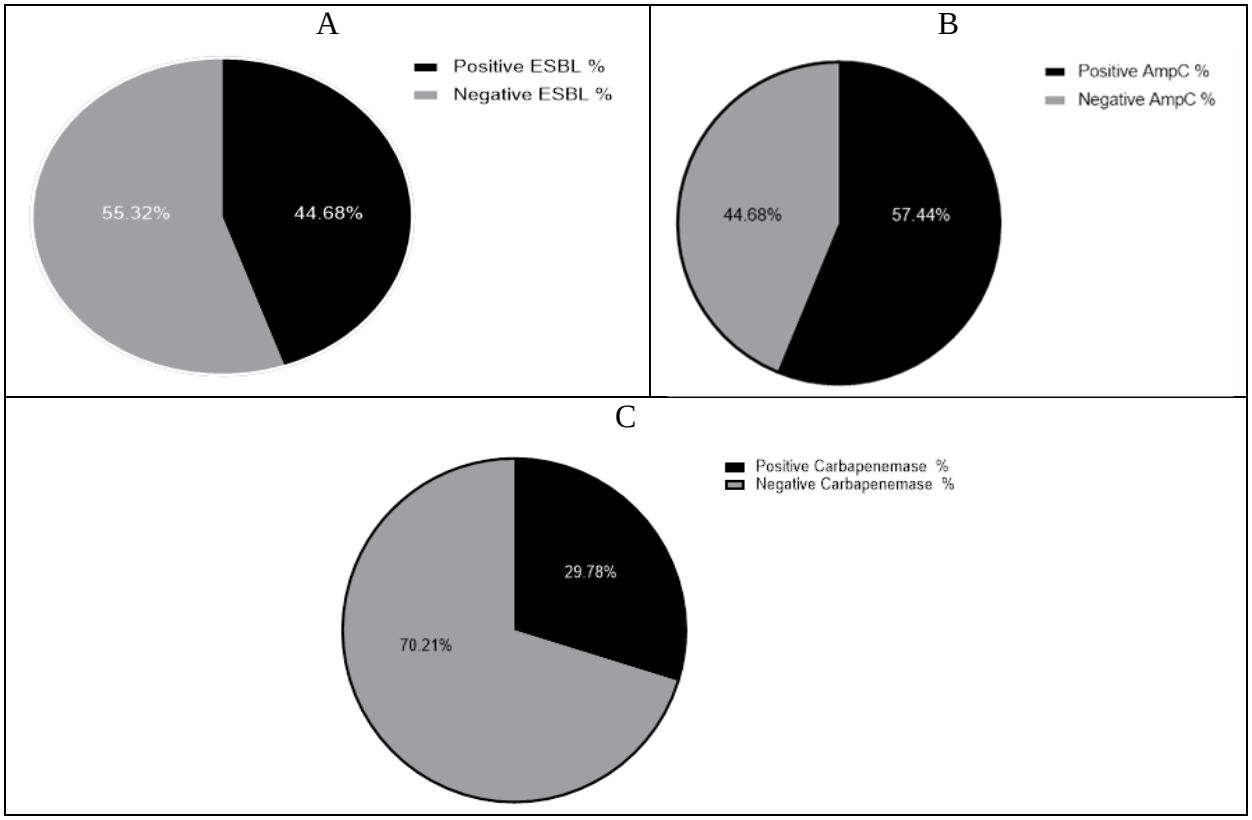

Fig 2. Antibiotics resistance mechanism % of Bcc isolates (A: % analysis of ESBL production by 47 BCC isolates, B: % analysis of AmpC β-lactamases production 47 BCC isolates, C: frequency of Carbapenemase production among 47 BCC isolates.

Conclusion

In conclusion, Early detection of Bcc resistance towards clinically applied antibiotics such as Ceftazidime, Ciprofloxacin, Carbapenem, and Chloramphenicol is thus an essential factor in reducing the morbidity and mortality of infections caused by Bcc. In addition, Proper microbial identification strategies should help identify and minimize the risk of Bcc contamination and infection outbreak inside healthcare facilities.

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Ethics approval and consent to participate

Not needed.

Conflict of interest

None of the authors report any conflict of interest

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