Phenotypic and genotypic typing of *Burkholderia cepacia* complex isolated from non-cystic fibrosis patients in two Kuwaiti hospitals

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BACKGROUND

Burkholderia cepacia complex (Bcc) is a group of opportunistic pathogens comprising of at least 20 closely related species that are phenotypically similar yet genetically distinct. Bcc is prevalent in nature and manmade environments due to its metabolic capacity. It most commonly affects patients with cystic fibrosis (CF). However, there are several cases of nosocomial Bcc infections in immuno-compromised patients with no history of CF. Lately, the life-threatening sporadic cases of infections caused by Bcc among ICU patients in Kuwaiti hospitals has been observed. The focus on Bcc species in this study is driven by their pathological significance and the lack of precise reports concerning their prevalence in Kuwait.

OBJECTIVES

- Phenotypic and molecular characterization of multidrug resistant Bcc isolates from Mubarak Al-Kabeer Hospital (MKH) and Amiri Hospital in Kuwait.
- Analyze the genetic relatedness (clonality) of Bcc isolates in Kuwait.
- Determine antimicrobial susceptibility of Bcc isolates collected from patients in both Mubarak Al-Kabeer Hospital and Amiri Hospital in Kuwait.

MATERIALS & METHODS

- A total of eight isolates were collected within six months from patients residing in Mubarak Al-Kabeer Hospital and Amiri hospital with no history of cystic fibrosis from different sites of infection. A QC strain was provided from the Allergy Centre (UK).
- Phenotypic identification (VITEK® 2 compact system and API20NE kit).
- Antimicrobial susceptibility using E-Test strips and agar dilution (for trimethoprim-sulfamethoxazole (TMP-sulfa)) to determine the MICs of antibiotics recommended by CLSI 2016 guidelines.
- Genotypic identification methods (PCR for *recA* gene, PCR-RFLP for *recA* , Pulsed-field Gel Electrophoresis (PFGE) and Multi-locus sequence typing (MLST)).

Antimicrobial agent	MIC results (µg/ml)								
	38	39	70	1317	1386	493	QC	75	76
Ceftazidime	8	>256	6	3	3	4	6	16	8
Meropenem	4	>32	2	2	0.75	4	3	8	>32
Levofloxacin	1.5	2	1.5	3	>32	6	2	1.5	2
Minocycline	8	8	2	4	8	>16	8	8	8
Chloramphenicol	>256	>256	32	48	32	64	32	64	8
Trimenthoprim/ Sulfamethoxazole	2/38	2/38	>0.5/9.5	1/19	> 8/152	1/19	>0.5/9.5	>0.5/9.5	>0.5/9.5

Table 1: Minimum inhibitory concentration (MIC) interpretation for Bcc isolates: Green = susceptible, orange = intermediate, red = resistant.

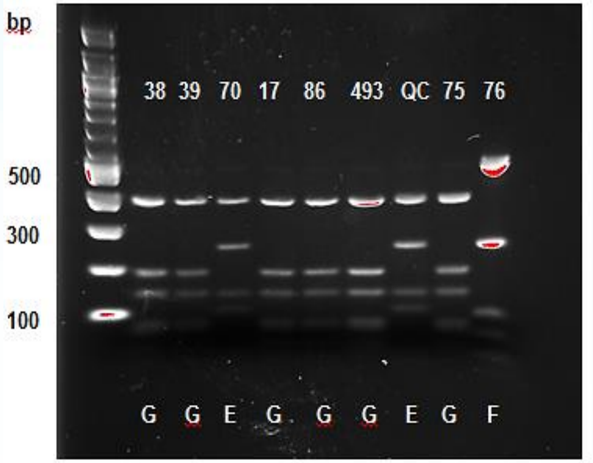

Figure 1: *Hae* III RFLP patterns of the *recA* gene amplified from Bcc strains isolated from clinical samples. G= *B. cenocepacia* (genomovar IIIa), **E**= *B. cepacia* (genomovar I), **F**= *B. multivorans* (genomovar II)

RESULTS

All isolates were identified as Bcc and produced a PCR product of 1000 bp Bcc-specific *recA* gene. Three different patterns were observed by PCR-RFLP for *recA.* Results of PFGE using *Spe* I restriction endonuclease illustrated the genomic diversity of the isolates. MLST profiles demonstrated different sequence types (ST). The not identified ones (NI) were submitted to PubMLST database to assign a new ST. Interpretation of MIC values showed all isolates were resistant to at least one group of antibiotics: ceftazidime (n=2), meropenem (n=3), minocycline (n=7), levofloxacin (n=3), TMP-sulfa (n=1), and chloramphenicol (n=8).

CONCLUSION

This is the first study performed to characterize the Bcc strains in Kuwait. The results will contribute to better understanding of Bcc bacteria and the genetic relatedness of Kuwaiti isolates to other regions of the world.

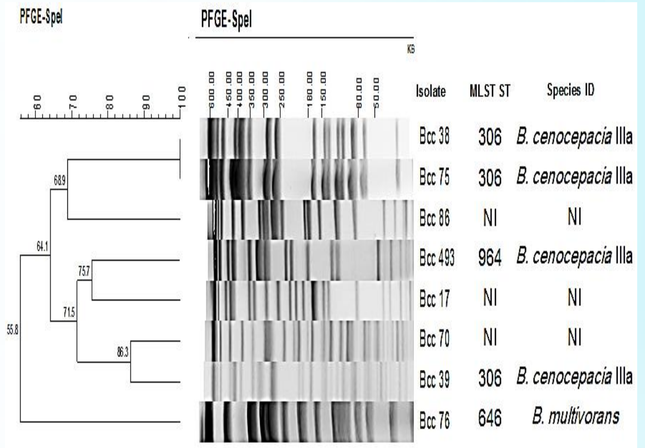

Figure 2: Pulsed-field gel electrophoresis profiles of Bcc isolates. Relationship between banding patterns after digestion with ***Spe* I** showing the percentage similarity the clusters of isolates generated by BioNumerics software v.7.1

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