Molecular Identification and Tetracycline's Resistance Genes among *Burkholderia cepacia* Isolates

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ABSTRACT
Burkholderia cepacia has an inherent ability to decrease the activity of antibiotics. This study aimed to screen tetracyclines and diagnose genes among *B.cepacia* isolated from different sources using Polymerase Chain Reaction (PCR). the results showed that among 950 specimens, 31 isolates were identified as *B. cepacia* isolates, and 700 of specimens exhibit bacterial growth compared with 250 no bacterial growth. Results were showed 380 return to female and 320 was return to male. The results showed a high level of resistance to each of the antibiotics ceftazidine (CAZ), trimethoprim /sulfamethoxazole (SXT) and meropenem (MEM), 27 (87%), 26 (83.87%), and 24 (77.41%) respectively. While minocycline (MIN) showed high efficacy against bacteria, with a resistance rate of 6 (19.35%). Results of PCR amplification showed high spreading of *Tet-A* and *Tet-B* among *B. cepecia* isolates which reached 24/31(77.41%) respectively, while *tet-D* and *tet-E* were absent among *B. cepecia* isolates in this study.
Keywords*:* Antimicrobial agents, *Burkholderia cepacia*, Tetracycline

INTRODUCTION
Burkholderia cepacia complex, often known as Bcc, is a significant opportunistic non-fermenting Gram-negative bacillus (NFGNB) that causes widespread infections in immunocompromised persons (Sethi *et al*., 2020). It is able to infect people as well as plants, and it may live in a variety of different conditions, such as soil or water. In humans, *B. cepacia* is mostly an opportunistic pathogen that may cause a variety of illnesses, including lung infections, in people who have chronic granulomatous disease or cystic fibrosis (Ho *et al*., 2021).
Antimicrobial resistance, often known as AMR, poses a significant danger to the health of people all over the world. Morbidity and mortality are both increased, and it is connected with substantial economic expenses because to the load it places on health care systems. Infections with multidrug-resistant bacteria, commonly known as multi-drug resistant (MDR) bacteria, have significant consequences not only for clinical but also for economic outcomes (Ikhimiukor *et al*., 2022). *Burkholderia cepacia* complex depends on various survival strategies, one of which is efflux pumps, which force biocides out of the cell or make them inactive so that the cell may continue to grow. This may be shown in their capacity to cause widespread contamination of both non-sterile and sterile medications, which can result in respiratory infections that pose a major danger to one's life as well as other serious health hazards (Daddy Gaoh *et al*., 2022). So this study aimed to molecular investigation of some tetracycline's resistance genes among *B.cepacia* isolated from different sources.

MATERIALS AND METHODS
Patients and Specimens Processing
The present study included 950 obtained from different clinical sources randomly for patients suffering different diseases included Burn, wound, diabetes foot ulcer, urine, throat and tissue were attended to main hospitals and burn centers in both Al-Najaf City and Baghdad City /Iraq, as well as clinical laboratories in Al-Najaf City-Iraq within the period of several months starting in

February September 2022. Using cotton swabs all specimens were streaked on MacConkey Agar and incubated aerobically at 37^{o}C overnight under sterile conditions (Collee *et al*.,1996).

Diagnosis of *B. cepacia* Isolates

Every suspected gram-negative bacteria with of *B.cepacia* characteristics were streaked on MacConkey Agar Medium based on microscopic , morphological, oxidase, and motility as well as some main biochemical tests (MacFaddin, 2000).

Monoplex PCR using to detect *Burkholderia* species via specific primer (Bur). The final identification was performed using the automated Vitek-2 compact system using ID-GP cards.

Antimicrobial agents susceptibility testing

According to the Kirby-Bauer technique, the antibacterial drugs ceftazidine (CAZ, 30 g), trimethoprim / sulfamethoxazole (SXT, 1.25/23.75 g), minocycline (MIN, 30 g), and meropenem (MEM,10 g) were tested on Mueller-Hinton agar for 31 clinical isolates of *B. cepacia* (Bauer *et al*., 1966). In order to prepare the inoculation of all of the isolates, the overnight growth of each of the tested isolates was first suspended in sterile normal saline that had been adjusted to a 0.5 McFarland standard tube. Discs sold in stores that contain antibacterial agents were tested. The zone diameters were interpreted in accordance with the instructions provided by the Clinical Laboratory Standards Institute (CLSI) guide (CLSI, 2021).

DNA extraction and PCR assay

Following the instructions provided by the manufacturing company, a genomic DNA extraction micro kit (Favorgen, South Korea) was used to collect all of the nucleic acids for 31 clinical isolates of *B. cepacia*. This was done in accordance with the manufacturer's protocol. After ensuring the integrity of the whole DNA sample by storing it in a deep freezer set to -20 degrees Celsius, a PCR analysis was carried out in order to test for the genes listed in Table 1. The equipment for gel documentation was employed for the migration of PCR amplification (bands) at 1% agarose, and then the bands were dyed with ethidium bromide at a concentration of 0.5 g/ml thereafter. The product of Tet-B gene of *B. cepacia* were sent to South Korea to done sequence by Macrogen company. The web site (http://www.ncbi.nlm.nih.gov/) was applied to alignment Tet-B gene sequence between some local *B. cepacia* isolates with other international strains.

Table (1): Primer Sequence and condition

Gene name	Primer Sequence 5' to 3'	Annealing (^{o}C)	Size of product(bp)	Reference
Bur -F	GARAAGCAGTTCGGCAA	57	385	Payne *et al*., 2006
Bur -R	GAGTCGATGACGATCAT			
Tet A-F	TTGGCATTCTGCATTCACTC	55	494	Ma *et al*., 2007
Tet A-R	GTATAGCTTGCCGGAAGTCG			
Tet B-F	CAGTGCTGTTGTGTCATTAA	55	571	Ma *et al*., 2007
Tet B-R	GCTTGGAATACTGAGTGTAA			
Tet-D-F	ATTACACTGCTGGACGCGAT	57	1070	Schmidt., *et al*., 2001
Tet-D-R	CTGATCAGCAGACAGATTGC			
Tet E-F	TATTAACGGGCTGGCATTTC	55	544	Ma *et al*., 2007
Tet E-R	AGCTGTCAGGTGGGTCAAAC			

RESULTS AND DISCUSSION

Patients and bacterial growth

Results of this study showed among 950 non-duplicated patient was 700 (73.68%) bacterial growth compared with 250 (26.31%) no bacterial growth. the results of biochemical tests , Vitek-2 system and PCR (figure,1), showed that among 950 specimens, 31 isolates were identified as *B. cepacia* isolates. A local study done in Hilla City by Abbas (2017) found the percentage of *B. cepecia* was 15 /787(1.7%) from sputum specimens compared with 263/878(30%) no growth.

The results of the PCR showed high efficiency in determining the genus of bacteria, and this is important and necessary to shorten the time of diagnosis, this matter is consistent with Fu *et al*., (2022), who indicated that the use of the PCR technique in the rapid and accurate detection of *B. cepacia* complex is vital and important to start effective treatment in a timely manner to avoid major deaths resulting from infections with this bacteria. In the same respect, according to bacterial growth in the Sexes , results were showed 380 (71.69%) return to female and 320(76.19%) was return to male. However, table (2) showed the rate of *B. cepacia* isolates were 18 isolates obtained from female compared with 13 isolates were obtained from male. Zlosnik *et al*., (2015) mention that the female sex as a risk factor for the result in infection by *B. cepecia* complex isolates.

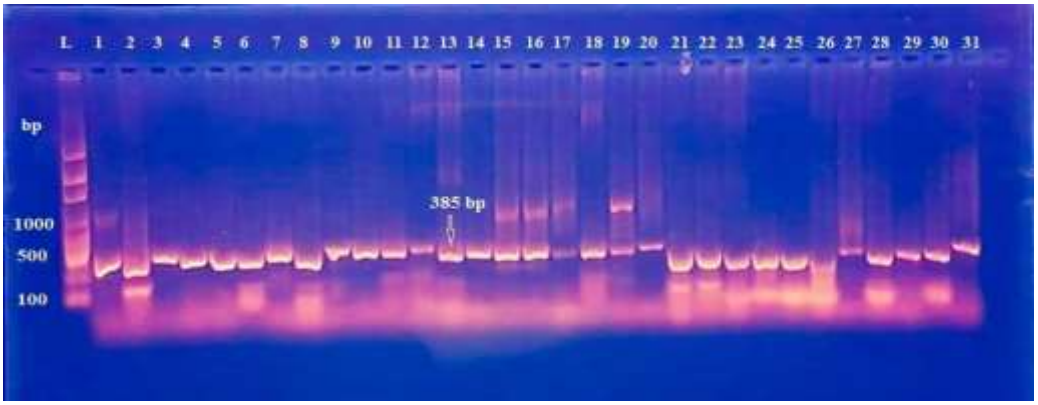

Figure (1): PCR amplification of *Bur* gene among total DNA of *B. cepacia* isolates

Table (2): Distribution of *B. cepacia* isolates among Female and male based on the Sex.

Sex	Total Specimens	specimens' growth		specimens' no growth		*B.cepacia*	
		Number	%	number	%	number	%
Female	530	380	71.69	150	28.30	18	3.39
Male	420	320	76.19	100	23.8	13	3.09
Total	950	700	73.68	250	26.31	31	3.26

Antibiotic susceptibility of *B. cepacia* isolates

The results of the effectiveness of the antibiotics recommended by CLSI showed variable efficacy in their effect on *B. cepacia* isolates (table, 3). The bacteria showed a high level of resistance to each of the antibiotics ceftazidine(CAZ), trimethoprim/sulfamethoxazole (SXT) and meropenem (MEM), 27 (87%), 26 (83.87%), and 24 (77.41%) respectively. According to a study done by Martina *et al*.,(2019) in Argentina they mention that a total of 53% and 46% of *Burkholderia* species were inhibited by ceftazidime and meropenem respectively, while Tseng *et al*., 2014 who reported (65%) of *B. cepacia* sensitivity to ceftazidine. At the same time, the antibiotic minocycline (MIN) showed high efficacy against bacteria, with a resistance rate of 6 (19.35). Peeters *et al*., (2009) reported that drugs of Meropenem, and ceftazidime were active against *B. cepacia* complex recorded 39.5% , while minocycline recorded 15.8% . Abbas, (2017) who reported that the susceptibility of *B. cepacia* to trimethoprim-Sulfamethoxazole and ceftazidine were (73.3%) and (66.7%) respectively. Many studies showed that bacteria have a natural

resistance to antibiotics, which could be due to the efflux pump or chromosomal -lactamases, but drugs like meropenem, trimethoprim-sulfamethoxazole, ceftazidime, and minocycline are effective against the *B. cepecia* complex (Tseng *et al*., 2014, Sfeir, 2018).

Table (3): Antibiotic susceptibility of *B. cepacia* isolates

Antibacterial agent	Resistance No.(%)	Intermediate No. (%)	Sensitive No. (%)
CAZ	27 (87%)	1 (3.2%)	3 (9.67%)
MIN	6 (19.35%)	9 (29.03%)	16 (51.61%)
MEM	24 (77.41%)	2 (6.45%)	5 (16.12%)
SXT	26 (83.87%)	3 (9.67%)	2 (6.45%)

Detection of tetracycline genes among *B. cepecia* isolates

Results of PCR amplification showed high spreading of *tet-A* and *tet-B* among *B. cepecia* isolates which reached 24/31(77.41%) for both *tet-A* and *tet-B* as well as sequence of tet-B gene revealed high rate of matching among local *B. cepecia* isolates with other international strain (figure 2, 3 and 4). In the same respect, *tet-D* and *tet-E* were absent among *B. cepecia* isolates and not detect in present study. Results of PCR showed 19 isolates have both *tet-A* and *tet-B* genes , while 10 isolates have one gene either *tet-A* or *tet-B* genes and only 2 isolates don't have *tet-A* and *tet-B* genes. The continuous competition between antibiotics and various bacterial infections is a serious problem that usually leads to the generation of new bacterial strains that are resistant to these antibiotics, which increases their danger to society. This phenomenon is often exacerbated by the rapid transfer of antibiotic resistance genes between Gram-negative bacteria (Behroozian *et al*., 2023).

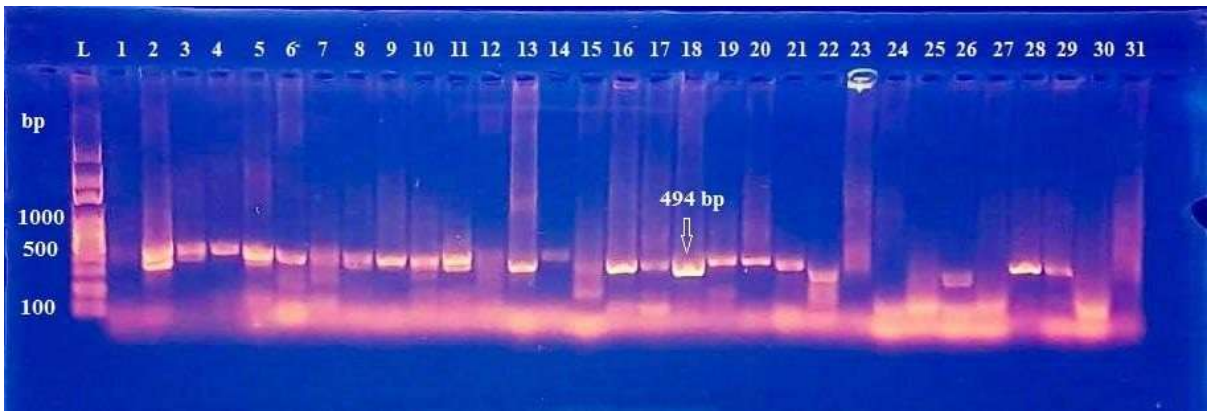

Figure (2): PCR amplification of *Tet-A* gene among total DNA of *B. cepacia* isolates (no., 12, 23, 24, 25, 27, 30, and 31 were negative bands)

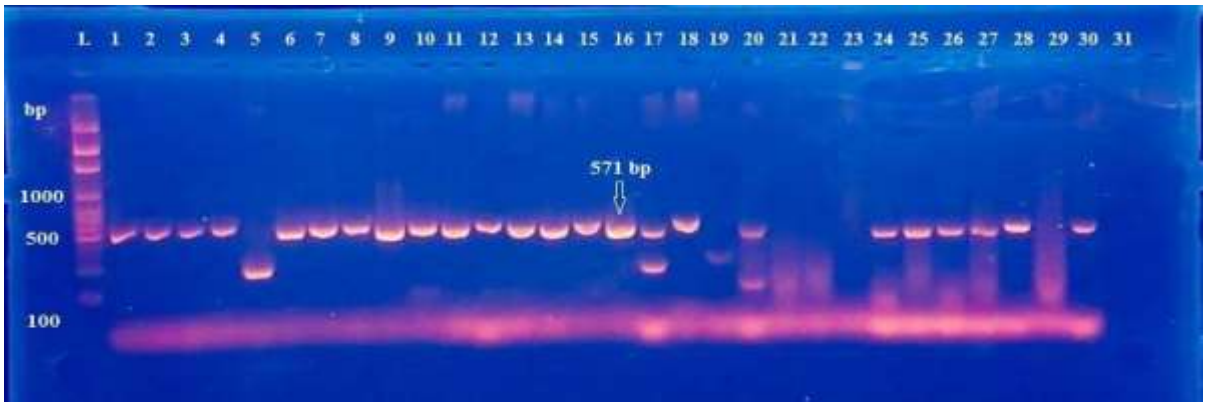

Figure (3): PCR amplification of *Tet-B* gene among total DNA of *B. cepacia* isolates (no., 5, 19, 21, 22, 23, 29, and 31 were negative bands)

Score	Expect	Method	Identities	Positives	Gaps
301 bits(770)	6e-100	Compositional matrix adjust.	151/154(98%)	151/154(98%)	0/154(0%)

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Query  2    ALWMLYLGRLLSGITGATGAVAASVIADTTSASQRVKWFGWLGASFGLGLIAGPIIGGFA  61
            ALWMLYLGRLLSGITGATGAVAASVIADTTSASQRVKWFGWLGASFGLGLIAGPIIGGFA
Sbjct  17   ALWMLYLGRLLSGITGATGAVAASVIADTTSASQRVKWFGWLGASFGLGLIAGPIIGGFA  76

Query  62   GEISPHSPFFIAALLNIVTFLVVMFWFRETKNTRDNADTEVGVETQSNSVYITLFKTMPI  121
            GEISPHSPFFIAALLNIVTFLVVMFWFRETKNTRDNADTEVGVETQSNSVYITLFKTMPI
Sbjct  77   GEISPHSPFFIAALLNIVTFLVVMFWFRETKNTRDNADTEVGVETQSNSVYITLFKTMPI  136

Query  122  LLIIYFSAQLIGHIPATEWVLFTENRFGCNSMMV  155
            LLIIYFSAQLIG IPAT WVLFTENRFG NSMMV
Sbjct  137  LLIIYFSAQLIGQIPATVWVLFTENRFGWNSMMV  170
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Figure (4): Alignment of amino acid sequence of *TetB* gene between *B. cepacia* isolates no.8 with *Escherichia coli* strain (Sequence ID: ACR19212.1 **)**

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