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Antibiotic Susceptibility Patterns of *Burkholderia cepacia* Isolated from Different Clinical Specimens

للنشر في مجلة جامعة بابل- العلوم الصرفة والتطبيقية المجلد (الرابع والعشرون) العدد (السادس) من المجلة ٢٠١٦.

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تحول المراسلات الى/ سكرتير التحرير - مجلة جامعة بابل - جامعة بابل - الحلة - محافظة بابل

Antibiotic Susceptibility Patterns of *Burkholderia cepacia* Isolated from Different Clinical Specimens

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Abstract:

In this study fifteen isolates of *Burkholderia cepacia* were isolated from (878) different clinical specimens collected from patients admitted to Al-Hashemyia General Hospital in Hilla city. The isolates were identified depending on their morphological properties and biochemical reaction, and underwent to Vitek 2 Compact system to confirm diagnosis. It was found that the highest percentage of isolation was from sputum specimens (53.3%). Antibiotic susceptibility patterns were studied, and the results revealed that imipenem was the most effective antibiotic against *B. cepacia* with susceptibility percentage (93.3%) followed by chloramphenicol (86.7%), erythromycin, trimethoprim –Sulfamethoxazole and levofloxacin with (73.3%). They exhibited moderate sensitivity to ciprofloxacin, ceftazidine (66.7%), tetracycline (60%), while *B. cepacia* isolates showed high resistance to gentamycin and oxacillin with (86.7%), (93.3%) respectively. *B. cepacia* isolates showed double and multiple resistance, and resistogram profiles were ranging from (6.7-33.3%), and showed commonest multiple drug resistance among inpatients *B. cepacia* isolates.

Keywords: *Burkholderia cepacia*, Antibiotic sensitivity**.**

الخلاصة:

تم في هذه الدراسة عزل ١٥ عزلة عائدة لبكتريا *Burkholderia cepacia* من مجموع ٨٧٨ عينة مختلفة مأخوذة من المرضى المراجعين الى مستشفى الهاشمية العام في محافظة بابل. شخصت العزلات جميعها بالاعتماد على الفحوص الزرعية والبايوكيميائية وثم خضعت العزلات الى جهاز Vitek 2 Compact system لغرض التأكد من تشخيصها، وكانت اعلى نسبة للعزل من عينات القشع (53.3%) . درست انماط الحساسية للمضادات الحياتية. اظهرت النتائج أن اكثر المضادات تاثيرا هو imipenem بنسبة حساسية (93.3%)، كما وجد حساسية للكلورومفينكول بنسبة (86.7%)، ونسبة (73.3%) لكل من erythromycin, trimethoprim –Sulfamethoxazole and levofloxacin، واظهرت العزلات حساسية متوسطة بنسبة (66.7%) لكل من ciprofloxacin, ceftazidine ونسبة (60%) للتتراسيكلين tetracycline في حين اظهرت العزلات مقاومة عالية لكل من gentamycin وoxacillin بنسبة (86.7%) و (93.3%) على التوالي. اظهرت العزلات مقاومة ثنائية ومتعددة ، وتراوحت نسب هياة المقاومة المتعددة من (6.7%–33.3%) ، وكانت هياة المقاومة المتعددة اكثر شيوعا في عزلات بكتريا *B. cepacia* المعزولة من المرضى الراقدين في المستشفى.

الكلمات المفتاحية: بكتريا *Burkholderia cepacia* ، الحساسية للمضادات الحياتية.

Introduction:

Burkholderia cepacia is non fermenting gram-negative, aerobic, motile bacillus, previously known as *Pseudomonas cepacia*, belong to the family Burkholderiaceae (Clontz, 2009). Originally, *B. cepacia* was described as a phytopathogen and isolated from soft-rotting disease of onion, fruits, vegetables, various soil, and water (Goldman and Klinger, 1986). Recently, *B. cepacia* has emerged as an opportunist pathogen, causing serious nosocomial infections among hospitalized and immunocompromised patients (Speert, 2002). *B. cepacia* causes an important pulmonary infections in patients with cystic fibrosis, chronic granulomatous disease and can lead to fatal systemic complications (Chaparro *et al*., 2001; Selina *et al*., 2012). In addition, *B. cepacia* has been increasingly implicated in community acquired infections, and recognized as causing infections among immunocompetent individuals such as pharyngeal infections (Fajardo *et al*., 2004), suppurative chronic otitis media, brain abscess (Hobson *et al*., 1995), pneumonia (Belchis *et al*., 2000; Bayram *et al*., 2011), bacteremia (Wong *et al*., 1991; Lu *et al*., 1997), and pediatric neck infections (Sousa *et al*., 2011).

B. cepacia is endowed with weak pathogenic potential (Lu *et al*., 1997). However, its ability to survive in nutritionally limited environments, tolerance a variety of physical conditions, high transmissibility between patients within and without hospitals, and its high resistance to many antimicrobial agents contributes to its ecological success, and its persistence in both community and hospital settings (Govan *et al*., 1993; Mahenthiralingam *et al*., 2008).

The characteristic multi-drug resistance of *B. cepacia* to antimicrobial agents plays a role in the increased prevalence. *B. cepacia* develops resistance by various mechanisms like antibiotic efflux pumps, biofilm formation, impermeability of the cell wall, alterations of intracellular targets and inactivation of drugs or enzymic degradation (Wigfield *et al*., 2002). In our hospitals, absence of precise reports about prevalence of *B. cepacia* infections which due to lack of consciousness and difficulty in diagnosis by routine clinical laboratories was the prompt to study the prevalence of *B. cepacia* in various clinical specimens, and to determine the susceptibility pattern of these isolates to different antibiotics.

Materials and Methods

Collection of specimens

A total of 878 different clinical specimens was collected from patients admitted to Al-Hashemyia General Hospital in Hilla city during a period extended from January to December 2014. The study included outpatients and inpatients of both sexes with different ages.

The specimens were included 118 sputum specimens, 94 blood specimens, 87 throat swabs, 382 urine specimens, 41 vaginal swab, 99 ear swabs, 45 stool specimens and 12 wound swabs. The specimens were collected by standard methods as mentioned in (Forbes *et al*., 2007).

Identification

The collected specimens were cultured on MacConkey's agar and nutrient agar, then incubated aerobically and at 37ºC for 24-72hrs for primary isolation. Non lactose fermented colonies on MacConkey's agar were selected and sub cultured to obtain pure culture. The morphological characteristics of the growing colonies including size, shape, colour, pigmentation and odor were recorded. The isolates were identified through biochemical tests according to (MacFadden, 2000; Forbes *et al*., 2007). Confirmative diagnosis for *B. cepacia* isolates was done by using Gram-negative GN identification card automated Vitek2 Compact system.

Antibiotic susceptibility test

Antibiotic susceptibility test of *B. cepacia* isolates were determined by Bauer Kirby's disc diffusion method according to (CLSI, 2012) (Clinical and Laboratory Standard Institute) guidelines. Mueller-Hinton plates were inoculated with a 0.5 McFarland standard suspension of *B. cepacia* organisms, and disks were placed. Zones of growth inhibition were recorded in millimeters after overnight incubation.

Results and Discussion

Isolation and identification

Incorrect or incomplete identification of an organism as *B. cepacia* cause a significant problem in infection control because of its high transmissibility among patients especially for the cystic fibrosis community (Chaparro *et al*., 2001). In the current study suspected *B. cepacia* isolates were characterized by morphological characteristics and biochemical testing **(Table 1)**. *B. cepacia* were identified by using automated Vitek2 Compact system as confirmatory test.

Even though molecular techniques are dependable and accurate method of identification, our hospitals do not have approach to such facilities for primary diagnostic microbiology. Vitek system provides accurate results for the identification of a challenging and diverse group of non-fermenters Gram-negative bacilli (Manji *et al*., 2014). Vitek ID-GNB cards correctly identified 92 to 100% of *B. cepacia* strains (van Pelt *et al*., 1991; Podin *et al*., 2013).

Table 1: Characterization of *B. cepacia*.

Character	Result
Gram stain	**-**
Shape	**rod**
O2 need	**Obligate aerobic**
Glucose oxidation	**+**
Catalase	**+**
Oxidase	**-**
Lysine decarboxylase	**+**
Ornithine decarboxylase	**+**
TSI	**K/K**
H2S production	**-**
Smell	**Dirt like odour**
Urease	**+**
Indole	**-**
MR	**-**
VP	**-**
Citrate	**+**
Gelatine liquefaction	**+**

Prevalence of *B. cepacia*

The prevalence of *B. cepacia* isolates in different clinical specimens examined over the study period was (1.7%) (**Table 2**). This level was not far away from other local study in Baghdad that reported a prevalence rate of 2.5 % of *B. cepacia* (Hassen *et al*., 2009). However this finding is relatively low when compared with global studies. In Iran, *B. cepacia* strains were isolated with (15.1%) of respiratory specimens (Eram *et al*., 2004). In Egypt, the percentage of *B. cepacia* complex isolates among the total clinical specimens was (23%) (Omar *et al*., 2015). In U.S.A., the percentage of *B. cepacia* isolates among the total clinical specimens was (18.9%) (Reik *et al*., 2005). In Spain, prevalence rate of *B. cepacia* strains was 14.9% among all positive samples (Medina-Pascual *et al*., 2012). In Ireland cystic fibrosis hospitals, prevalence of *B. cepacia* complex was 2.75% in 2004 and peaked at 7.6% in 2010, this was dropped to approximately 6% for the last three years (Keating and Schaffer, 2015). In China, a study recorded that *B. cepacia* accounts for approximately 3.81% of nosocomial infections (Ming *et al*., 2003).

There are some variables that influences the differences in prevalence of *B. cepacia* between studies, such as clinical specimens received for examination, study period, studied population, type of hospitals and geographical locations. One of the possible reasons for low prevalence of *B. cepacia* in our study is that most of clinical specimens collected from outpatients comparing to global studies that isolated *B. cepacia* from hospital acquired infections and cystic fibrosis hospitals.

Table 2: Number and percentage of *B. cepacia* isolates

Cultural result specimens	Number of isolates	%
Burkholderia cepacia	**15**	**1.7%**
Other bactereia	**600**	**68.3%**
No growth	**263**	**30%**
Total	**878**	**100**

The results showed that 53.3% of *B. cepacia* isolates were collected from male patients, while 46.7% of *B. cepacia* isolates were collected from female patients (**Figure 1**). *B. cepacia* infections were seen almost equally in both males and females, this is possible may due to fact that both man and women may represent workforce, so both are exposed to contaminated work environment outside house, and thus subjected for acquiring infectious diseases. This gender distribution was incompatible with (Keating and Schaffer, 2015) who reported that 70 % of *B. cepacia* isolated from male patients while 30% from females.

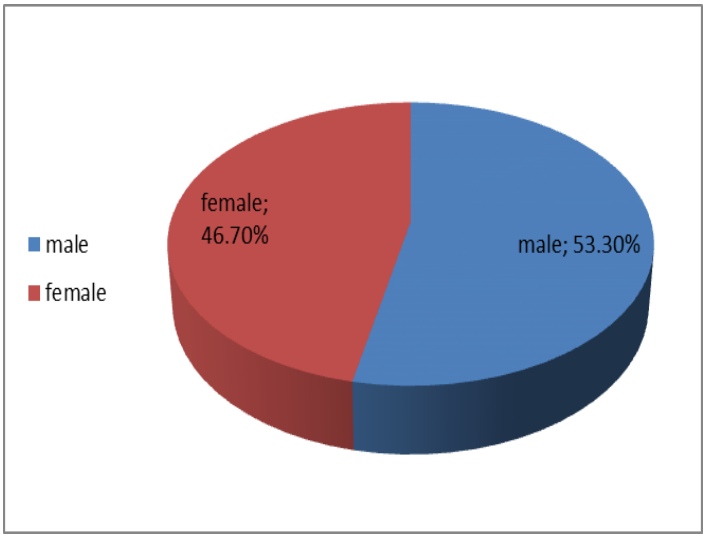

Figure 1: Distribution of *B. cepacia* isolates according to gender

The prevalence of *B. cepacia* infections according to age groups was also studied. It was shown that *B. cepacia* infections are distributed in all age groups with highest prevalence 40% of cases in older age group of 20-40 followed by 33.3% were in the age group of 41-60 , then 20% in the age group less 20, and only 6.7% were in elderly age group above 61 year (**Figure 2**).

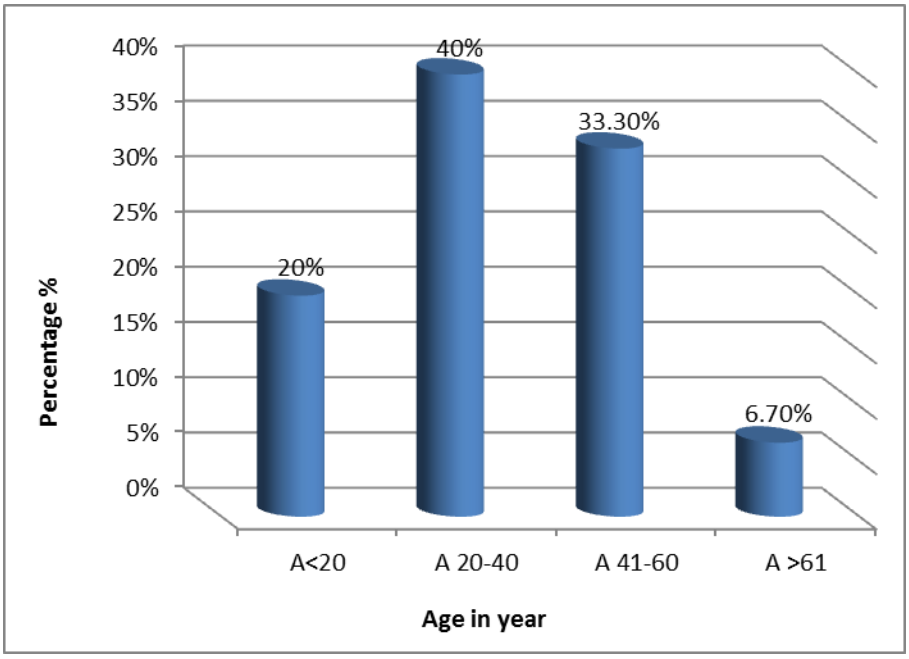

Figure 2: Distribution of *B. cepacia* isolates according to age

Results (**Figure 3**) demonstrated that percentage of *B. cepacia* infections was highest during spring season representing about 46.6% of infections all year along, followed by equal incidence in summer and winter seasons with about 26.7%% of infections, while autumn season shows complete absence of *B. cepacia* infections.

B. cepacia is environmental bacterium, many studies have reported that humans may possible acquire *B. cepacia* directly from natural environments (Fisher *et al*., 1993). Human pathogenic *B. cepacia* strains are not necessarily distinct from environmental soil strains (LiPuma *et al*., 2002). The most recent of these studies pointed out that *B. cepacia* type strain ATCC 25416T, isolated from rotting onions in the 1940s, was also isolated from sputum of cystic fibrosis patient in the UK. Similarly, *B. cenocepacia* strains, isolated from soil, was indistinguishable by several typing methods (pulsed-field gelelectrophoresis PFGE, genomic fingerprinting and repetitive extragenic palindromic rep PCR) from clinical *B. cenocepacia* isolates isolated from cystic fibrosis patients (Baldwin *et al*., 2007). Thus higher incidence of *B. cepacia* infections in spring may be possible explained by increasing human agricultural activities and contacting with soil during this season.

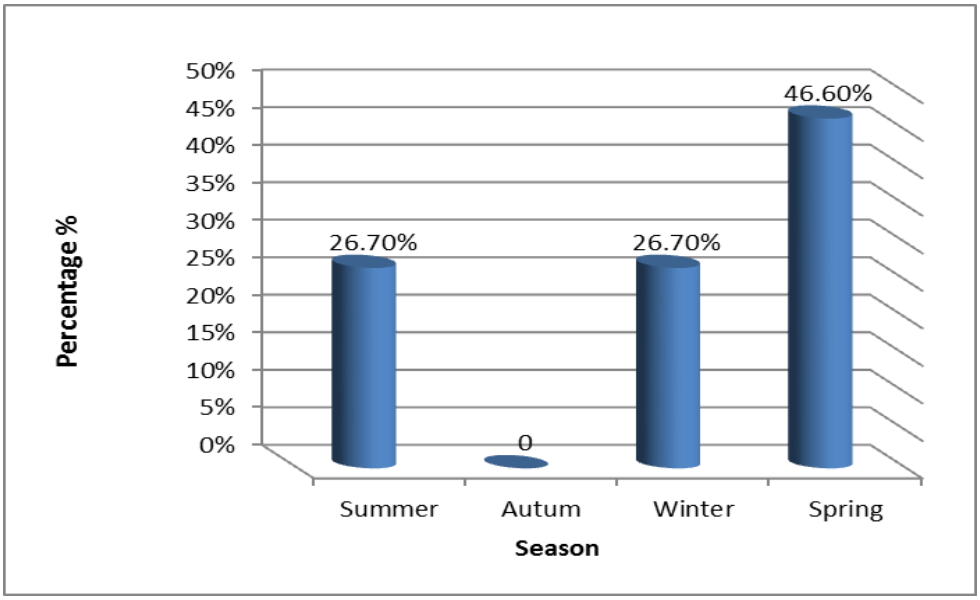

Figure 3: Distribution of *B. cepacia* isolates according to seasons

The present study revealed that the highest percentage of *B. cepacia* isolates were isolated from sputum specimens 8/15(53.3%) followed by throat swabs 5/15(33.3%), while the percentage of *B. cepacia* isolates from blood specimens was 2/15(13.3%). Results showed completely absence of *B. cepacia* isolates from urine specimens, stool, vaginal, ear and wound swabs (**Figure 4, table 3**).

However, other global studies reported higher rates of isolation of *B. cepacia* from specimens other than those in our study. In Egypt, the highest rate of isolation of *B. cepacia* was from pus 30/35 (85.7%) followed by sputum 4/35 (11.4%) and urine 1/35 (2.9%) (Omar *et al*., 2015). In Taiwan, significant higher proportion of *B. cepacia* isolates were recovered from blood (68.2%) followed by sputum (15.2%), and urine (4.6%), surgical wounds (3%), and pus (1.5%) (Tseng *et al*., 2014). In Latin America, found that 52/83 (62.7%) of *Burkholderia* spp. were from blood, 25/83 (30.1%) were from sputum, 3 (3.6%) were from skin and soft tissue infection and also 3 (3.6%) were from urine (Gales *et al*., 2005). In Iran, most *B. cepacia* strains were isolated from throat swabs (75%), and (25%) of isolates were from sputum (Eram *et al*., 2004). In Poland, a study on hospitalized patients reported that *B. cepacia* is responsible for (70.2%) of urinary tract infections, (7%) of surgical wound infections, and (8%) of CSF, while no *B. cepacia* were detected in sputum and blood (Gospodarek *et al*., 1997).

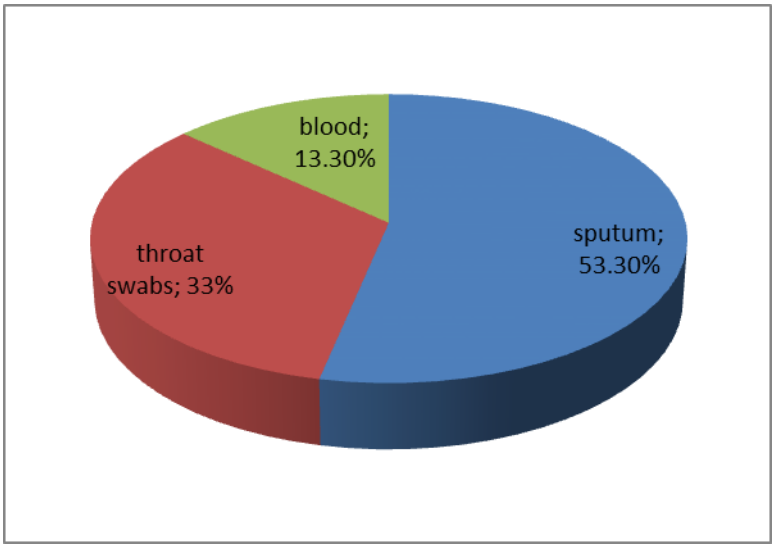

Figure 4: Percentage of *B. cepacia* isolates in different clinical specimens

According to type of specimens (**Table 3**), in sputum specimens, there were 8 *B. cepacia* isolates from 118 specimens and the isolates percentage was (6.8%), this level was higher when compared with local studies that indicated the percentage of *B. cepacia* isolates in sputum specimens was 3.64% and 1.6% respectively (Abdul-Latif, 2004; AL-Dahash *et al*., 2013).

The variations in the percentage of isolation between different studies could be due to the percentage of distribution of isolates which varied according to the place of clinical specimens collection, and number of clinical specimens, environmental factors and personal hygiene (Ogunseilan, 2005).

Table 3: Specimens profile and frequency of *B. cepacia* isolates

Types of specimens	**Number of Specimens (878)**	**Number of *B. cepacia* isolates (15)**	**The percentage of each isolation source (14.6%)**
Sputum	**118**	**8**	**6.8%**
Throat swab	**87**	**5**	**5.7%**
Blood	**94**	**2**	**2.1%**
Urine	**382**	**0**	**0**
Vaginal swab	**41**	**0**	**0**
Ear swab	**99**	**0**	**0**
Stool	**45**	**0**	**0**
Wound	**12**	**0**	**0**

Ten different antibiotics representing different families were tested on *B. cepacia* isolates for studying there antimicrobial sensitivity pattern. Our results (**Figure 5**) revealed that imipenem was the most effective antibiotic against *B. cepacia* with percentage (93.3%) of antimicrobial sensitivity . Imipenem is a broad-spectrum carbapenems antibiotics. Beta-lactam ring of this antibiotic is resistant to hydrolysis by most β-lactamases. In addition, high sensitivity may due to uncommon uses of imipenem in our country.

The result was in accordance with studies reported that *B. cepacia* showed sensitivity to imipenem (Hassen *et al*., 2009; Omar *et al*., 2015; Dizbay *et al*., 2009). However, disagrees with study of (Di-yong *et al*., 2009) which reported 41.1% resistance to imipenem of *B. cepacia.* Carbapenem resistance was reported to be as high as 48 -89% among *B. cepacia* isolated from nosocomial infections with cystic fibrosis (Araque-Calderon *et al*., 2008; Medina-Pascual *et al*., 2012).

Imipenem was followed by chloramphenicol with percentage (86.6%) of antimicrobial sensitivity of *B. cepacia*. A study found that *B. cepacia* isolated from patients with malignancy demonstrated sensitivity more than (60%) to chloramphenicol (Roy *et al*., 2014). However, disagrees with (Omar *et al*., 2015), who reported sensitivity rates (37.1%). Routine exposure of bacteria only to newly developed antibiotics removed resistance against older out of use antibiotics and most present *B. cepacia* isolates were sensitive to these outdated agents like chloramphenicol.

In the present study, the percentage of sensitivity to erythromycin and trimethoprim -Sulfamethoxazole was (73.3%). Majority of isolates in current study were isolated from outpatients that showed more susceptibilitiy to the antibiotics.

Sensitivity of *B. cepacia* to trimethoprim –sulfamethoxazole was in agreement with (Tseng *et al*., 2014), while disagree with (Omar *et al*., 2015; Gautam *et al*., 2009) who reported sensitivity rates (0%, 43.6%) respectively .

Regarding quinolones sensitivity, results indicated the percentage of sensitivity to levofloxacin, ciprofloxacin was 73.3%, 66.7% respectively. A study reported that *B. cepacia* isolated from patients with community-acquired *Burkholderia* pneumonia revealed sensitivity to quinolones (Bayram *et al*., 2011). Another study in Taiwan reported that *B. cepacia* isolated from patients demonstrated (80%) sensitivity to levofloxacin (Tseng *et al*., 2014). This disagree with (Hassen *et al*., 2009; Dizbay *et al*., 2009; Di-yong *et al*., 2009; Omar *et al*., 2015) who reported higher levels of resistance to ciprofloxacin, levofloxacin.

In respect to cephalosporins, *B. cepacia* isolates showed sensitivity percentage (66.7%) to ceftazidine. This result is in agreement with

(Tseng *et al*., 2014) who reported (65%) of *B. cepacia* sensitivity to ceftazidine. Other study showed (73.7%) of patients received ceftazidime were cured (Avgeri *et al*., 2009). However, disagrees with other studies (Hassen *et al*., 2009 and Di-yong *et al*., 2009) which found sensitivity rates(20%, 44%) respectively. Tetracycline in the present study showed sensitivity percentage (60%). This result was higher than reported by (Omar *et al*., 2015) (5.8%) and less than (Gautam *et al*., 2009) (76.7%).

In respect to aminoglycosides, the results showed (13.3%) of *B. cepacia* isolates were sensitive to gentamicin. This result agrees with study of (Hassen *et al*., 2009) which found sensitivity rates (20%). The results revealed that high resistance (93.3%) to β-lactam (oxicillin).

B. cepacia are resistant to many antimicrobial agents. *B. cepacia* resistance to β-lactam antibiotics most commonly results from combination of impermeability and constitutively expressed or inducible chromosomal β-lactamases or efflux pumps. Less commonly, plasmid-mediated β-lactamases of the TEM class (cephalosporinases) confer resistanse to β-lactam antibiotics(Tseng *et al*., 2014).

B. cepacia have intrinsic resistance to aminoglycosides which due to A lack of binding sites on the lipopolysaccharide, reduced outer membrane permeability, and efflux pumps (Cox and Wilkinson, 1991). In addition, efflux pump system confers resistance to chloramphenicol, tetracycline, and ciprofloxacin . *B. cepacia* resistance to trimethoprim results from production of dyhydrofolate reductase or acquisition of efflux pumps that confer cross resistance to fluoroquinolones and chloramphenicol (Burns *et al*., 1989).

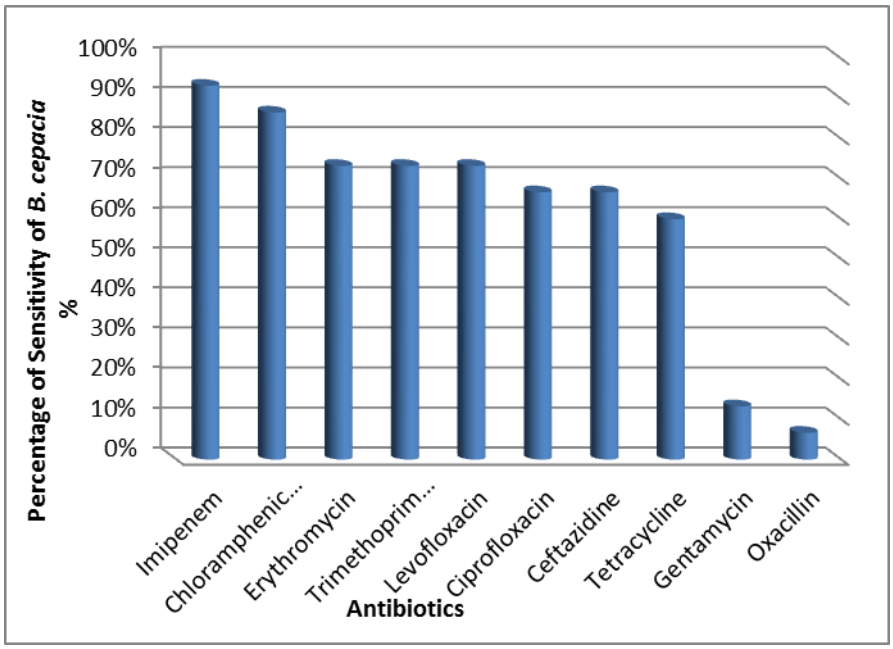

Figure 5: Antibiotic sensitivity of *B. cepacia* isolates

In current study, the resistogram profiles of the *B. cepacia* **(Table 4)** ranging from (6.7%-33.3%) and revealed that all the 15 *B. cepacia* isolates were distributed among 10 different resistance profiles. The resistogram profiles were as double, triple, and multiple . The largest multiple resistogram profile **OX, GN, TE, IMP, CAZ, CIP, SXT, E, LEV, C** was isolated from sputum of inpatients, In addition, 2 isolates shared resistogram pattern **OX, CAZ, TE, CIP, E, GN, LEV** were isolated from blood of two inpatients suffering from septicemia, where other *B. cepacia* isolates with different resistance profile were isolated from outpatients. It has been noted in different studies that nosocomial acquired bacterial isolates tend to be more resistance than community acquired isolates, and frequently showed resistance and cross resistance to many antibiotics. The continuous selective pressure of regularly used antibiotics may be explained this resistance (Gospodarek *et al*., 1997; Dizbay *et al*., 2009; Bayram *et al*., 2011).

Table 4: Resistogram profiles of *B. cepacia* isolates

Resistance profile		Antibiotics	Number	Total %
Double resistance		**OX, GN**	**5:15**	**33.7%**
		OX, TE	**1:15**	**6.7%**
		TE, SXT	**1:15**	**6.7%**
Triple resistance		**OX, TE, GN**	**1:15**	**6.7%**
		OX, E, GN	**1:15**	**6.7%**
		OX, CIP, GN	**1:15**	**6.7%**
Multiple resistance	**5**	**OX, CAZ, SXT, GN, C**	**1:15**	**6.7%**
	6	**OX, CAZ, CIP, SXT, GN, LEV**	**1:15**	**6.7%**
	7	**OX, CAZ, TE, CIP, E, GN, LEV**	**2:15**	**13.3%**
	10	**OX, GN, TE, IMP, CAZ, CIP, SXT, E, LEV, C**	**1:15**	**6.7%**

Oxacillin (OX), Imipenem (IPM), Gentamycin (GN), Erythromycin (E), Chloramphenicol (C),) Tetracycline (TE), Ceftazidine (CAZ), Levofloxacin (LEV), Ciprofloxacin (CIP), Trimethoprim –Sulfamethoxazole (SXT).

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