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Antimicrobial Resistance in Bacterial Pathogens isolated from clinica different blood specimens

Mohsen Hashim Risan[1], Shams Ahmed Subhi[2], Ghydaa H Al-Jeboury[3]
[1] College of Biotechnology, Al-Nahrain University, Iraq
[2] Al-Turath University College, Iraq
[3] Biotechnology Research Center, Al-Nahrain University, Iraq
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Abstract
The current study aimed to determine the antimicrobial resistance. One hundred and five of clinical sample were collected from Central Teaching Hospital of paediatric and Medical city / Educational laboratory, from patients (Male, Female and infant) of age range between. Out of one hundred and five clinical samples were screened, only seventy isolates were collected as 17 identified as Staphylococcus aureus, 12 identified as Klebsiella pneumonia depending on cultural, microscopical and biochemical characteristics. The remaining isolates were identified as Staphylococcus spp., E.coli and Acinetobacter baumannii, pseudomonas aeruginosa, Enterobacter spp., Proteus spp. Regarding to the patients gender, it was found that infant had a tendency to get infection more than males and female when 30 (28.57 %) of patients were males, 33 (31.42 %) females and 42 (40%) infant. Moreover, the age group ≤ 1year were most subjected to the infection of bloodstream infection. Antibiotic susceptibility of bacterial isolates were tested against thirteen of commonly used antibiotic was determined through disc-diffusion method, the result exhibits that the most reliable antibiotic can used as drug is imipenem because mostly all bacterial isolates were sensitive to it and also not used before, in addition to amikacin could be used also as a drug because it given intramuscular and pain so it has low resistance of bacterial isolates.

Keywords: Bacteremia, Antimicrobial, BSI, Iraq

Introduction
Bacterial infection (bacteremia) is the most common cause of sepsis, more than 45% of BSI are caused by single bacterial species (House, 2010) [25]. Blood can get infected by microorganisms such as bacteria, which are pathogens that can introduce in normally sterile environments (blood), such opportunistic pathogens e.g. Staphylococcus aureus, Escherichia coli, Acinetobacter spp and klebseilla pneumonia (Hansen, 2012) [23]. The main cause of BSI are gram-negative and gram-positive bacteria, which possession their virulence factors that help them to attack the blood stream (Shrestha *et al*., 2007) [50]. About 80 - 90% of BSI caused by type of Enterobacteriaceae called klebsiella pneumonia and gram positive bacteria Staphylococcus aureus (Orsini *et al*., 2012) [39]. Klebsiella pneumonia is an opportunistic pathogen and this property is related to possessing capsule, which responsible for increasing acute disease, such as septicemia, pneumonia, urinary tract infection, and soft tissue infections (Vuotto *et al*., 2014) [56]. Staphylococcus spp can grow best on blood agar plates, but Staphylococcus aureus which are common species, their colonies are frequently surrounded by zones of clear beta-haemolysis. Staphylococcus aureus infections cause fatal disease in patients whose immune system are impaired or weaekend as the bacterium is highly aggressive and able to invade and destroy tissues, which are capable of producing toxins that cause the most common life threating disease toxic shock syndrome (TSS), also bacteraemia and sepsis (Otto, 2014) [42]. Antimicrobial act by targeting important bacterial functions such as cell wall synthesis (beta-lactams and glycopeptides), protein synthesis (aminoglycosides, tetracyclines, macrolides, lincosamides, chloramphenicol, mupirocin and fusidic acid), nucleic acid synthesis (quinolones), RNA synthesis (rifampin), and metabolic pathways such as folic acid metabolism (sulphonamides and trimethoprim) (Wright, 2005; Tenover *et al*., 2006; [53] Alekshun and Levy, 2007) [4]. This study was carried out to determine the Antimicrobial resistance of Staphylococcus aureus, Klebsiella pneumonia, E. coli, Acinetobacter baumannii, Pseudomonas aeruginosa, Enterobacter spp and Proteus spp isolated from clinical different blood samples were collected from Central Teaching Hospital of paediatric and Medical city / Educational laboratory In Baghdad /Iraq.

Materials and Methods Antibiotics
The following antibiotic discs were used during this study:

Table 1: Used Antibiotic Discs

Antibiotic	Abbreviation	Concentration (µg / disk)	Source/ origin
Amikacin	AK	30	Bioanalyse/Turkey
Ampicillin	AMP	10	Bioanalyse
Azithromycin	AZM	15	Bioanalyse

Ceftazidime	CAZ	10	Bioanalyse
ciprofloxacin	CIP	5	Bioanalyse
Imipenem	IPM	10	Bioanalyse
Ceftraiaxone	CTR	10	Bioanalyse
Vancomycin	VAN	30	Bioanalyse
Chloramphenicol	C^+	30	Bioanalyse
Gentamycin	GEN	30	Bioanalyse
Tetracycline	TE	30	Bioanalyse
Clindamycin	CD	20	Bioanalyse

Collection of samples

One Hundred and five samples of blood were collected from patients suspected of having blood stream infection and certain clinical symptoms. These samples were collected from Medical city Hospital / educational laboratories and Central Teaching Hospital of pediatric in Baghdad. Samples were collected from different age groups and genders.

Blood samples

Blood is drawn from patients by using a syringe (5 ml). It is immediately transferred to a clean sterilized brain heart infusion broth tube, the blood is then allowed to clot for at least 10 to 15 minutes at room temperature, then kept in an incubator for 18 hours for further laboratory investigations (Tille *et al*., 2013) [54].

Blood Culture

The blood specimens were inoculated on blood agar, McConkey agar and chocolate agar plates by direct streaking method using a loop to deliver a loopful of the blood specimens. After incubation overnight at 37ºC the bacterial growth is examined, if there were no growth, the plates were re-incubated for another 24 hours before they were considered as a negative culture (Novak-Weekley and Dunne, 2016) [38].

Isolation and identification of bacteria

Bacteria have been isolated from pure colonies and cultured on blood, McConkey, and chocolate agar then isolated bacteria were examined microscopically by using Grams stain technique for referred to as Gram-positive or Gram-negative bacteria. The identification tests include cultural, morphology, and physiological characteristics of each bacterial isolates were done (Brown, 2005) [13].

Identification of Morphological characteristics

Colonies of the bacterial isolates that cultured on blood agar and MacConky media were described according to their shapes, color, diameter, odor, and other characteristics (Macfaddin, 2000) [35].

Microscopic Examination

The microscopic examination includes two procedures, gram stain, and capsule stain (Atlas *et al*., 1995).

Biochemical tests

The following biochemical tests were performed for the identification of bacteria. These tests were carried out according to (Forbes *et al*., 2002) [18], includes (Catalase test, Blood hemolysis, Oxidase, Indole test, Citrate utilization and Urease production test (Atlas, 2010) [7], and Culturing on Eosin methylene blue (EMB) agar, Motility test (Garrtiy, 2005), and Mannitol fermentation (This test is specific for Staphylococcus aureus) (Collee *et al*., 1996) [14].

Antibiotic susceptibility test

Susceptibility of bacterial isolates to different antibiotics was examined according to the standard disk diffusion method National Committee for Clinical Laboratory Standards (NCCLS), (2000) as follows:

1. Five ml of sterile brain heart infusion broth was inoculated with 0.1 ml of the fresh culture of Klebsiella pneumoniae isolate or Staphylococcus aureus, E. coli, Acinetobacter baumannii, Pseudomonas aeruginosa, Enterobacter spp and Proteus spp and incubated at 37°C for 4 hrs, then serial dilutions were prepared, and 0.1 ml of the fourth dilution (10^{-4}) was spread on Muller-Hinton agar plate in different three planes by rotating the plate approximately 60°C each time to obtain an even distribution of the `inoculum.
2. Plates were then placed at room temperature for 30 min to allow the absorption of excessive moisture.With a sterile forceps, antibiotic disks were placed on the surface of the medium (5-8 disks/ plate) and incubated at 37 Cº for 24hs.
3. After incubation diameters of the inhibition zones were measured and compared with standards of the National Committee for Clinical Laboratory Standards.

Results and Discussion

Isolation of bacteria

Blood samples from a total of 105 clinical different blood samples were collected from Central Teaching Hospital of paediatric and Medical city / Educational laboratory, In Baghdad /Iraq. Table (2) samples were collected from different age groups and gender. Seventy (66.6 %) were clinical blood positive samples, while the rest (35) were negative blood samples (33.3 %).

Table 2: Total number of samples used for the isolation of bacteria

Clinical sample	Positive (growth)	Negative (no growth)
(105)	70	35
Percentage	66.66%	33.33%

Incidence of blood stream infection (BSI)

Blood samples from a total of 105 clinical different blood samples were collected, their ages ranging from(infant: 1 day - 12 month), (adult: 19 - 75 years). The results have showed that 70 (66.6%) of blood samples contained heavy bacterial growth while 35 (33.3%) of samples had no bacterial growth as demonstrated in table (2). This study agrees with cases in Pakistan Latif and Ahmed (2017) when they reported that incidence of BSI in patient were 97.6% while disagreement in

India Waghmare *et al*., (2015) when they reported that incidence of BSI in patients were 18.6%.
The relationship of BSI with the age of patients was investigated in this study and the patients were grouped into three categories according to their age as shown in table (3).

Table 3: Distribution of incidence of Bsi in relation to age of patients

Age group	(19-75) years		(1 day- 12 month)
Specimen	Male	Female	Infant
105	30	33	42
Percentage	28.57%	31.42%	40%

The results (table 3) have showed that infant had a tendency to get infection more than male and female with BSI, when the age of adult ranged 19 - 75 year in which compared with infant age ranged 1 day - 12 month, that's cause of high fever and focal infection seen in children or infant, this result agrees with Babay *et al*., 2005 and Abbas *et al*., 2014, which announced paediatric infant patients get more infection with BSI, while disagree with Gedik *et al*., 2014 that concluded female can get more infection with BSI.

Identification of bacterial isolates
Several morphological, physiological and biochemical tests were made to identify bacterial isolates. Seventeen isolates were obtained from one hundred and five samples. Results showed that Klebsiella spp. constitute 17.1% (12 isolates), and identified as K. pneumoniae, Staphylococcus spp. Constitute 15.7% (11 isolates), Staphylococcus aureus constitute 24.2% (17 isolates). The other bacterial isolates were constituted Escherichia coli 14.2% (10 isolates), Acinetobacter baumannii 14.28% (10 isolates), Proteus spp. 2.82% (2 isolates), pseudomonas aeruginosa 7.14% (5 isolates), and Enterobacter spp. 4.28% (3 isolates). Figure (1), illustrates the percentages of each bacterial species found in the collected samples.

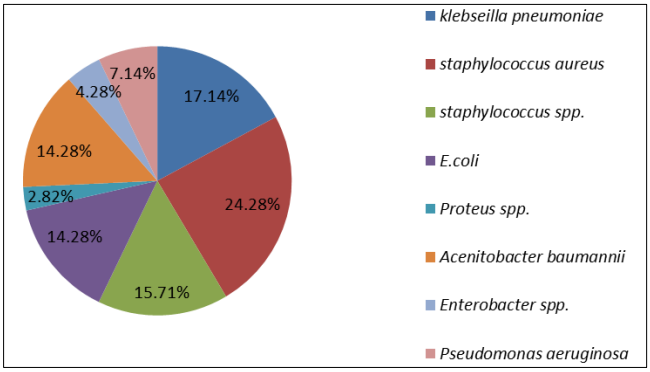

Fig 1: Bacterial isolates obtained from blood samples**.**

Bacterial isolates were identified according to their cultural, microscopical and biochemical characteristics that were in agreement with Holt *et al*. (1994), Atlas *et al*. (1995) and Collee *et al*. (1996) [14].

Biochemical Tests
The biochemical tests were used for further identification of bacterial isolates. Table (4) showed that all isolates of Klebsiella, Staphylococcus, Proteus, pseudomonas, and Enterobacter were negative result for indole while positive result for E. coli. In the indole test, ability to hydrolyze tryptophan to indole is a characteristic of certain enteric bacteria possessing the enzyme tryptophanase, an enzyme that decomposes amino acidtryptophan to indole, pyruvic acid and water. Indole negative bacteria was not produced tryptophanase, so that when Kovac's reagent was added to a broth free of indole, a red ring will not be formed at the top of the broth (Collee *et al*.,1996) [14]. Utilization of citrate is one of several important physiological test used to diagnose members of all Enterobacteriaceae except E.coli which negative for citrate, while Acinetobacter baumannii and Pseudomonas aeruginosa are positive for citrate. Citrate in simmon citrate medium is important to detect weather the bacteria isolates able to grow on it as a unique carbon and energy source. In addition, Simmon's medium also contains bromothymol blue as a pH indicator. Klebsiella is produced CO2, it reacts with components of the medium to produce an alkaline compound, the alkaline pH turns the pH indicator (bromthymol blue) from green to blue, reflecting it as positive citrate test (Macfaddin, 2000) [35]. In Kligler Iron Agar (KIA) test, it differentiates the genera of Enterobacteriaceae from each other based on their carbohydrate fermentation patterns and H2S production. KIA slants contain 1 % lactose and 1 % glucose. The pH indicator (phenol red) changed the medium color from orange-red to yellow in the presence of acids. KIA also contains sodium thiosulfate, a substrate for H2S production, and ferrous sulfate that produces black precipitate to differentiate H2S producing bacteria from others. Results (table 4) showed that Klebsiella, isolates turned the color of both the slant and butt, which produced acidic slant (yellow) and acid butt (yellow) accompanied by gas production (bubbles formation), but without black precipitate formation, which indicates that lactose and glucose fermentation had occurred and no H2S was produced. These results agreed with those declared by Garrity (2005) [20]. E.coli isolates turned the color of both the slant and butt, which produced acidic slant (yellow) and acid butt (yellow) accompanied by CO_2 production but without black precipitate formation, which indicates that lactose and glucose fermentation had occurred and no H2S was produced, These results agreed with those declared by (Penalver *et al*., 2005) [43]. Proteus spp isolates turned the color of both the slant and butt, which produce acidic butt (yellow) and alkaline slant (red) accompanied by H_2S production (black precipitant) that indicates of glucose fermenting and non-lactose fermenting, These results agreed with those declared by (Saadabi *et al*., 2010) [49]. Acinetobacter baumannii isolates turned the colour of slant to alkaline butNo change bottom, No gas, No H_2S production that indicate non-lactose fermenting, These results agreed with those declared by (Hussein *et al*., 2013). Pseudomonas aeruginosa isolates turned the colour of slant and butt to alkaline without production of H_2S and gas that indicate non-lactose fermenting, these results agreed with those declared by (Tunç and Olgun, 2006) [55]. Enterobacter spp turned the color of both the slant and butt, which produced acidic slant (yellow) and acid butt (yellow) accompanied by gas production (bubbles formation) but without black precipitate formation, which indicates lactose fermentation and non-glucose fermentation had occurred and no H2S was produced,These results agreed with those declared by (Ng *et al*., 2007).

Table 4: Cultural, Microscopically, Physiological and Biochemical characteristics of different bacterial isolates

No.	Isolates test		K. pneumoniae	S. aureus	E. coli	A. baumannii	Proteus	P. aeruginosa	Enterobacter
1	Cell shape		Bacilli	Cocci	Bacilli	Coco-bacilli	Bacilli	Bacilli	Bacilli
2	MacConkey agar		LF	LNF	LF	LNF	LNF	LNF	LNF
3	Gram stain		-	+	-	-	-	-	-
4	Capsule stain		+	-	-	-	-	-	-
5	Motility		-	-	V	-	V	+	V
6	Urease		+	+	-	-	+	-	-
7	Indole		-	-	+	-	-	-	-
8	Citrate utilization		+	+	-	+	V	+	V
9	Kliglar iron agar (KIA)	H_2S	-	-	-	-	+	-	-
		CO_2	+	-	+	-	ND	+	-
		Acid	A/A	ND	A/A	K/K	A/K	K/K	A/A
10	Oxidase		-	-	-	-	-	+	-
11	Catalase		V	+	V	+	V	+	+
12	Mannitol fermenter		V	+	V	-	-	V	V
13	Coagulase		ND	+	-	ND	ND	-	-

(+) positive result, (–) negative result, (ND) Not determined, (K) alkaline, (A) acid, (V) variable result, (LF) Lactose ferment, (LNF) Lactose Non-ferment

Whereas urease test were positive for Klebsiella, Staphylococcus and negative for Enterobacter, proteus, E.coli, pseudomonas, and Acinitobacter (Forbes *et al*., 2002) [18]. Urease enzyme catalyzes the breakdown of urea, and the bacteria that can produce this enzyme is able to detoxify the waste products and to drive metabolic energy from its utilization which change the medium color from yellow to purple-pink, indicating urease positive test. Klebsiella can produce urease enzyme and gives urease positive test (Atlas *et al*., 1995). In the motility test, Klebsiella isolates were non-motile. The movement of the growth away from the stab line or a hazy appearance through the semisolid medium indicates that the bacteria are motile. But the linear growth means negative result a property which Klebsiella is characterized by Gwendolyn (1988). While Acinetobacter baumannii isolates were non-motile, these results agreed with those declared by (Hussein *et al*., 2013). Moreover E.coli, Pseudomonas aeruginosa, Enterobacter spp and Proteus spp isolates virable motile (O'toole and Kolter, 1998; RÖmling, 2005; Pomorski *et al*., 2007; Berg, 2008). Another hand in oxidase test Klebsiella, E.coli, proteus, Enterobacter, isolates were oxidase negative and catalase positive or negative result (Bernere and Farmer, 2005), where else Pseudomonas aeruginosa isolates were oxidase positive and catalase positive (PHE, 2015), while Staphylococcus aureus isolates were oxidase negative and catalase positive (Orwin *et al*. 2003), and finally Acinetobacter baumannii isolates were oxidase negative and catalase positive (Doughari *et al*., 2011). The coagulase test is specific to differentiate Staphylococcus aureus from other species and genera which is positive (Kateete *et al*., 2010).

Distribution of bacterial isolates according to the samples:

Results presented in table (5) show that out of 105 clinical samples, 17 (15.7%) Staphylococcus aureus, 12 (17.1%) Klebsiella pneumoniae, 11 (24.2%) Staphylococcus spp., 10 (14.2%) E. coli, 10 (14.2%) Acinetobacter baumannii, 5 (7.14%) pseudomonas aeruginosa, 3 (4.28%) Enterobacter spp. and 2 (2.82%) Proteus spp. isolates were recovered.

Table 5: Types and numbers of bacterial isolates obtained from samples

No.	Bacterial isolates	Number of isolates
1	Klebsiella pneumoniae	12 (17.14%)
2	Staphylococuus aurues	17 (24.28%)
3	Stapylococcus spp	11 (15.71%)
4	Escherichia coli	10 (14.28%)
5	Proteus spp	2 (2.82%)
6	Acinetobacter baumannii	10 (14.28%)
7	Pseudomonas aeruginosa	5 (7.14%)
8	Enterobacter spp	3 (4.28%)
	Total	70 (66.66%)

Results revealed that S. aureus and K. pneumoniae (17, 12 isolates respectively) was the dominant among all other species of bacteria. This result was in agreement with the report documented by Waghmare *et al*, (2015) and Karki (2010) [29] whom found that those two species was the most frequently occurring among other species, when its account for 29 % of Klebsiella pneumoniae and 65% of Staphylococcus aureus isolated clinically.

Antibiotic resistance of bacterial isolates

Antimicrobial resistance (AMR) is now recognised as a serious global threat. The control of AMR requires a national, international coordination across hospitals, region and borders on both the reduction/adaptation of antibiotic use and the implementation of specific precautions to avoid cross-transmission of multi-drug resistant organisms. Antimicrobial therapies, such as antivirals and antibiotics have played an

Essential role in the treatment of infections in humans and animals and have significantly improved public health for the last 100 years. Without antimicrobials, serious infections like pneumonia and tuberculosis could not be treated, complex medical interventionssuch as organ transplantations would not be possible due to the high risk of hospital acquired infections, and care of premature babies would be a serious challenge (EUCIC, 2017).

The recent rise in resistance to these critical medicines is therefore extremely worrisome without effective antimicrobials; much of the progress made in fighting infectious disease globally and prolonging life would be lost. For that reason, global action has been launched to fight antimicrobial resistance (WHO, 2015). The main causes that leads to loss the active of the drug is genetic mutation, over exposure in environment, use of antibiotic in animal (poetry, cows) and finally the abused of drugs in medical (EUCIC, 2017). Susceptibility of the 70 isolates of bacteria was examined towards 13 different antibiotics using disc diffusion method recommended by the National Committee for Clinical Laboratory Standards (NCCLs) guideline. Results presented in table (6) shows that coagulase negative Staphylococcus spp. (CoNS) isolates were have high resistance to gentamicin (72.7%) and ceftriaxone (72.7%). This returns to the massive use of these two drugs that leads to develop of resistance, and that could also returns to the bad behaviour of doctors and medical practices that lead the drug lost in its effectiveness. This result was in agreement with Koksal *et al*., (2009), that concluded the high resistance for gentamycin (90%) and sensitive to ampicillin (91%) while disagree with Duran *et al*. (2012) [16].

Table 6: Antibiotic resistant of Staphylococcus spp. Isolates

Antibiotic	R	P	S	P
Amikacin	1	9.09%	10	90.9%
Ampicillin	3	27.27%	8	72.72%
Azithromycin	6	54.54%	5	45.45%
Ceftazidime	2	18.18%	9	81.18%
Ciproflaxacin	5	45.45%	6	54.54%
Gentamycin	8	72.72%	3	27.27%
Imipenem	4	36.36%	7	63.63%
Vancomycin	5	45.45%	6	54.54%
Clindamycin	2	18.18%	9	81.18%
Ceftriaxone	8	72.72%	3	27.27%
Chloramphenicol	6	54.54%	5	45.45%
Tetracycline	7	63.63%	4	36.36%

R: Resistant S: Sensitive P: percentage

Also the results presented in Table (7) shows that 17 Staphylococcus aureus isolates were have high resistance to chloramphenicol (76.4%) and has high sensitivity to amikacin (100%). This returns to the massive use of chloramphenicol drug that leads to become highly resisted and lost its effectiveness, while ampicillin was also documented as the third common drug to be resisted because of its abused in influenza, regarding sensitivity its was notice that the most common sensitive drug is 1) amikacin because it's given intramuscular and painful so it's give highly sensitivity, 2) imipenem because of new drug that's not used before. Azithromycin and clindamycin and in the third place ceftazidime, gentamycin and vancomycin that's because of less use of these drugs in medical practices they retain by their sensitivity till now fortunately. As the antibiotic becomes more widely used and becomes more prevalent in the market as soon as antibiotic resistance increases. This result agree with Waghmare *et al*. (2015) and Ronat *et al*., (2014), which concluded that Staphylococcus aureus has sensitive to amikacin (92.9%) while did not agree with Kitara *et al*., (2011).

Table 7: Antibiotic resistant of Staphylococcus aureus isolates

Antibiotic	R	P	S	P
Amikacin	0	0%	17	100%
Ampicillin	10	58.8%	7	41.1%
Azithromycin	6	35.2%	11	64.7%
Ceftazidime	7	41.1%	10	58.8%
Ciproflaxacin	12	70.5%	5	29.4%
Gentamycin	7	41.1%	10	58.8%
Imipenem	6	35.2%	11	64.7%
Vancomycin	7	41.1%	10	58.8%
Clindamycin	6	35.2%	11	64.7%
Ceftriaxone	8	47.0%	9	52.2%
Chloramphenicol	13	76.4%	4	23.5%
Tetracycline	9	52.2%	8	47.0%

R: Resistant S: Sensitive P: percentage

The Results presented in table (8) shows that 12 Klebsiella pneumoniae isolates were has high resistance to the group of cephalosporin (ciproflaxacin, ceftazidime and ceftiaxone) because of the over use in medical from the first generation to the last one, among the other antibiotics group of aminoglycoside such as amikacin, gentamycin, and clindamycin they was the reliable drug for all gram-negative bacteria but few years later now the overuse leads to lost its activity and become resisted. In the other side ampicillin has been used forbidden in last few years, in which returns some of sensitivity. One other hand also has resistance for ceftazidime and ciprofloxacin (100%, 91% respectively). This result agrees with Prabhash *et al*. (2010) which concluded that all klebsiella pneumoniae isolates has resist the group of cephalosporin, while the result disagree with Karki *et al*. (2010) [29] who notice that sensitive to amikacin in 91.7%.

Table 8: Antibiotic resistant of Klebsiella pneumoniae isolates

Antibiotic	R	P	S	P
Amikacin	8	66.6%	4	33.3%
Ampicillin	4	33.3%	8	66.6%
Azithromycin	6	50%	6	50%
Ceftazidime	12	100%	0	0%
Ciproflaxacin	11	91.6%	1	8.3%
Gentamycin	7	58.3%	5	41.6%
Imipenem	0	0%	12	100%
Vancomycin	5	41.6%	7	58.3%
Clindamycin	10	83.3%	2	16.6%
Ceftriaxone	8	66.6%	4	33.3%
Chloramphenicol	7	58.3%	5	41.6%
Tetracycline	5	41.6%	7	58.3%

R: Resistant S: Sensitive P: percentage

The Results presented in Table (9) shows that 10 E. coli isolates were having high sensitive to amikacin (100%) and less sensitive to gentamycin (90%). This result agrees with Karki *et al*. (2010) [29]. who notice that E.coli has high sensitive to amikacin in 74.7% and less sensitive to gentamycin also agrees with Shyamala *et al*., (2012) [51] who shows that E.coli has high sensitive to amikacin while the result disagrees with Kayange *et al*. (2010).

Table 9: Antibiotic resistant of E. coli isolates

Antibiotic	R	P	S	P
Amikacin	0	0%	10	100%
Ampicillin	9	90%	1	10%
Azithromycin	8	80%	2	20%
Ceftazidime	10	100%	0	0%
Ciproflaxacin	7	70%	3	30%
Gentamycin	1	10%	9	90%
Imipenem	0	0%	10	100%
Vancomycin	0	0%	100	100%
Clindamycin	4	40%	6	60%
Ceftriaxone	6	60%	4	4%
Chloramphenicol	5	50%	5	50%
Tetracycline	5	50%	5	50%

R: Resistant S: Sensitive P: percentage

Results presented in table (10) shows that 10 Acinetobacter baumannii isolates were having high resistance to chloramphenicol and less resist to ciprofloxacin (100%, 90%respectively). This could returns to highly virulent and pathogenic of the bacterium. Imipenem has high active drug against Acinetobacter baumannii because of the new generation that never being used before. This result agree with Abdulla *et al*. (2015) [2] who notice that this bacterium has resist to chloramphenicol and ciprofloxacin in (93%,80%)respectively. while disagrees with Hujer *et al*. (2006) [26] showed that A. baumannii strains isolated from military and civilian personnel injured.

Table 10: Antibiotic resistant of Acinetobacter baumannii isolates

Antibiotic	R	P	S	P
Amikacin	7	70%	3	30%
Ampicillin	6	60%	4	40%
Azithromycin	5	50%	5	50%
Ceftazidime	8	80%	2	20%
Ciproflaxacin	9	90%	1	10%
Gentamycin	7	70%	3	30%
Imipenem	0	0%	10	100%
Vancomycin	3	30%	7	70%
Clindamycin	6	60%	4	40%
Ceftriaxone	9	90%	1	10%
Chloramphenicol	10	100%	0	0%
Tetracycline	9	90%	1	10%

R: Resistant S: Sensitive P: percentage

Results presented in table (11) shows that 5 Pseudomonas aeruginosa isolates were have high resistance to the most of drugs such as amikacin, ampicillin, azithromycin, ceftazidime, ciproflaxain,ceftriaxone, and chloramphenicol, while it has high sensitive to imipenem and tetracycline, that cause of the high virulent and pathogenic of the bacterium and also low of isolates was obtained.This result were agree with Anjum and Mir (2010) [5] whom declared that P. aeruginosa has high resistance to ampicillin and ceftriaxone in (100%) but has high sensitive to imipenem (97%) followed by amikacin (79%), while the result disagrees with Rajat *et al*. (2012) [46], who clarify that this bacterium has resistant to imipenem in 14% while Sievert *et al*. (2013) [52] who notice that all strains of P. aeruginosa has highly sensitive to the carbapenem group of antibiotics like imipenem (78.57%).

Table 11: Antibiotic resistant of Pseudomonas aeruginosa isolates

Antibiotic	R	P	S	P
Amikacin	4	80%	1	20%
Ampicillin	5	100%	0	0%
Azithromycin	3	60%	2	40%
Ceftazidime	5	100%	0	0%
Ciproflaxacin	4	80%	1	20%
Gentamycin	2	40%	3	60%
Imipenem	0	0%	5	100%
Vancomycin	1	20%	4	80%
Clindamycin	3	60%	2	40%
Ceftriaxone	4	80%	1	20%
Chloramphenicol	4	80%	1	20%
Tetracycline	0	0%	5	100%

R: Resistant S: Sensitive P: percentage

Results presented in table (12) shows that 3 Enterobacter spp. isolates were having high resistance to ampicillin, azithromycin, ceftazidime, ciproflaxacin, ceftriaxone, clindamycin, and tetracycline (100%, 66.6%, 100%, 100%, 100%, 66.6%, 66.6% respectively) because of Enterobacter species have become increasingly important nosocomial pathogens, however, resistance to cephalosporin's group often complicates the treatment of infection, as well as this bacterium has resist to other microbial agents during therapy, so the choice of appropriate antimicrobial agents is complicated, also the low isolate was obtained. In other hand Enterobacter spp. shows high sensitive to gentamycin, imipenem, amikacin, vancomycin and chloramphenicol in (100%, 100%, 100%, 66.6%, 66.6%) respectively. This result agrees with Kang *et al*. (2004) who clarify that 47% of Enterobacter spp has resisted to all cephalosporin's group, while disagrees with Boban *et al*. (2011) who observe that Enterobacter spp has sensitive to ceftazidime in 51.6%.

Table 12: Antibiotic resistant of Enterobacter spp. Isolates

Antibiotic	R	P	S	P
Amikacin	0	0%	3	100%
Ampicillin	3	100%	0	0%
Azithromycin	2	66.6%%	1	33.3%
Ceftazidime	3	100%	0	0%
Ciproflaxacin	3	100%	0	0%
Gentamycin	0	0%	3	100%
Imipenem	0	0%	3	100%
Vancomycin	1	33.3%	2	66.6%
Clindamycin	2	66.6%	1	33.3%
Ceftriaxone	3	100%	0	0%
Chloramphenicol	1	33.3%	2	66.6%
Tetracycline	2	66.6%	1	33.3%

R: Resistant S: Sensitive P: percentage

Proteus spp. remains susceptible to nearly all antimicrobials except tetracycline. Resistance does not appear to be a significant clinical factor, but 10%-20% of strains can acquire resistance to ampicillin and first-generation cephalosporins. Results presented in table (13) shows that 2 Proteus spp. isolates were having high sensetive to amikacin, imipenem and tetracycline (100%), while shows high resistance to azithromycin, ceftazidime, gentamycin, and vancomycin. The reason is due to of the high virulent and pathogenic of Proteus spp. and also low isolates was obtained. This result agree with Adamus-Bialek *et al*. (2013) [3]who clarify that all Proteus spp. has displayed a much higher sensitivity to amikacin and imipenem. while the result disagree with Biswas *et al*. (2014) [11] who notice that Proteus spp. has resisted to amikacin and imipenem from 80% to 100% respectively.

Table 13: Antibiotic resistant of proteus spp. Isolates

Antibiotic	R	P	S	P
Amikacin	0	0%	2	100%
Ampicillin	1	50%	1	50%
Azithromycin	2	100%	0	0%
Ceftazidime	2	100%	0	0%
Ciproflaxacin	1	50%	1	50%
Gentamycin	2	100%	0	0%
Imipenem	0	0%	2	100%
Vancomycin	2	100%	0	0%
Clindamycin	1	50%	1	50%
Ceftriaxone	1	50%	1	50%
Chloramphenicol	1	50%	1	50%
Tetracycline	0	0%	2	100%

R: Resistant S: Sensitive P: percentage

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