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Isolation, Identification and Antibiotic Susceptibility of pathogenic Bacteria Isolated from Clinical Samples

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Abstract: *150 Samples have been collected, through the period from January/2016 to June/2016 . This for isolation and identification of pathogenic gram negative bacteria. The samples were involve 50 burns samples for isolate Pseudomonas aeruginosa, 50 stool samples for isolate Escherichia coli and 50 urine samples for isolate Enterobacter cloacae and Proteus mirabilis. The isolates were identified by ten biochemical tests as well as the sensitivity and resistant were tested by thirteen type of antibiotics. These antibiotics explained different mode of action in their activity, were resistant, moderate and sensitive, some of them have significant differences when comparison together and some have no. The Trimethoprim, Amikacin, Aztreonam and Novobiocin have high activity against Ps. aeruginosa, E. coli, E. cloacae and P. mirabilis respectively, as well as the Aztreonam was more active when comparison the activity of these antibiotics together.*

Keyword: *Pathogenic gram negative bacteria, Resistant, Antibiotic, biochemical tests.*

I. Introduction

Gram negative bacteria play important role in infectious diseases, but the *Pseudomonas aeruginosa*, *Escherichia coli, Enterobacter cloacae* and *Proteus mirabilis* now become the broad spectrum species involved in nosocomial infections (Anuj Nautiyal *et al*., 2015).Antibiotics identified, action modes and bacterial resistance mechanisms have been researched topics in academia until recently in the pharmaceutical applications (Davies and Davies., 2010). Bacteria can be resistant one or more type of antibiotics, the mechanisms by which bacteria resist antibiotics include, alter the receptor for the antibiotic, decrease antibiotic amount that reaches the receptor by preventing entry or removal, antibiotic alteration or destroying and develop resistant metabolic pathways (Fisher and Mobashery., 2010).

The *Pseudomonas aeruginosa* are aerobic, motile gram negative rod bacteria worldwide distributed and found as skin flora and in the majority of environmental sources (Jabalameli *et al*., 2011). It is can survive in different environments such as aquatic and terrestrial and contributes strongly to nosocomial infections and infected of immunocompromised peoples (Eguchi *et al*., 2013). This bacteria has widespread antibiotic resistance among their clinical isolates, as well as considered as one of the most important bacterial agents associated with nosocomial infections (Mirsalehian *et al*., 2010), also known as the most common bacterial agents in the burn patients (Azimi *et al*., 2015). This bacteria causes invasive infections in burn and this lead to sepsis from invasive burn wound infection (Alhazmi., 2015).

The inherent antibiotics resistance mechanisms for *Pseudomonas aeruginosa* involve lower outer membrane permeability, increased expression of efflux pumps for different specificity and presence beta-lactamase give this bacteria resistance mechanisms to commonly used antibiotics (Strateva and Yordanov 2009). This is true for several opportunistic pathogenic bacteria like *Ps. aeruginosa* which causes number of chronic infections due to resistant to modern antimicrobial therapies (Gellatly and Hancock 2013). Whether in the clinic or in the natural environment this bacteria encounters multiple classes of traditional antibiotics, antiseptics and disinfectants (Murray *et al*., 2015).

The *Escherichia coli* are a microorganism that commonly live in the large intestine of humans and other warm blooded animals, this is the most prevalent facultative gram negative bacteria in the human fecal flora and usually inhabit the colon as commensal microorganism (Manikandan and Amsath., 2014). Some strains of this bacteria can cause disease inside and outside of the gastrointestinal tract (Manikandan and Amsath., 2014). This bacteria is used in the variety of fields in both the industrial and medical, also the most used bacterium in the technology of recombinant DNA (Yoo *et al*., 2009).

E. coli is has resistant to therapeutic levels of the first β-lactam that act on cell wall, and this because of its outer membrane is barrier to this type of antibiotics (Allocati *et al*., 2013). Also, this bacteria have resistant to several different types of antibiotics with distinct mechanisms of action (Johnson *et al*., 2012). The emergence and spread of resistant against multidrug for strains of *Escherichia coli* is complicating and become difficult in the treatment of several serious infections caused by this bacteria (Allocati *et al*., 2013). Family of *Enterobacteriaceae*, specillay of *Escherichia coli*. are the most frequent cause of hospital and this lead to community acquired infections (Pitout., 2012).

The use of antimicrobial agents in wrong ways for animals and humans treatment lead to the emergence and diffusion of the several antibiotic resistant strains of *Escherichia coli* that also capable to infect other humans through either direct contact with animals or through consumption of contaminated food with this bacteria (Ewers *at al*., 2012). *Escherichia coli* have ability to survive and adapt in different extra intestinal locations and to produce resistances between humans, animals and their products through environment by several transmission pathways (Ewers *at al*., 2012).

The *Enterobacter* belonging to the family of *Enterobacteriaceae* which is common Gram negative, rod shaped and non spore forming bacteria, live as facultative anaerobic and have been reported as important opportunistic and multi resistant bacterial pathogens and causes infection for humans (Davin-Regli and Pages., 2015). There are two main known species for this genus, which are *E. aerogenes* and *E. cloacae* have take non clinical significance as opportunistic bacteria and have emerged as nosocomial pathogens (Mezzatesta *et al*., 2012). *Enterobacter cloaca*e now is the most frequently observed in clinical isolate among *Enterobacter* species but, the pathogenicity and factors contributing in the disease caused by this bacteria still not understood (Davin-Regli and Pages., 2015). This bacteria has been associations with clinical samples particular in urines with property of antibiotics resistant (Pestourie *et al*.,2014). *E. cloacae* has several mechanisms for resistant of wide range of antibiotics (Davin-Regli and Pages., 2015). Various studies on antibiotic resistance *E .cloacae* which associated with low level susceptibility to several antibiotics by inducing the over expression of the efflux pump (Perez *et al*., 2012).

The *Proteus* is genus belonging to the family of *Enterobactericeae* and of gram negative bacteria, this is widespread in the environment and makes third as the cause of hospital acquired infections it frequently causes nosocomial infections of the urinary tract (Bahashwan., 2013). This genus consists of four species: *Proteus vulgaris*, *Proteus penneri*, *Proteus myxofaciens* and *Proteus mirabilis* (Penner *et.al*., 1984). The latter is the most causative agent of majority *Proteus* infections (Feglo *et al*., 2010) and the most one of bacteria that responsible for urinary tract infections (Nabeela *et al*., 2004).

Proteus mirabilis is generally susceptible to most antibiotics, including beta-lactams, aminoglycosides and imipenem but it has intrinsic resistance to nitrofurantoin, tetracycline and colistin (Hara *et al*., 2000). However, the multidrug resistant strains of *Proteus* species has also been reported worldwide (Singla *et al*., 2015). As well as has ability to resistant to several different types of antibiotics and called multi antibiotics resistant (Dadheech *et al*., 2015). In addition to including recent evidences of multidrug resistance *P. mirabilis* (Wong *et al*., 2013).

II. Materials and Methods

Isolation of pathogenic gram negative bacterial:.

Table:1. Percentage of all isolated

Bacterial type	Site of Collection	Number	No. of isolates	Samples (%)
Ps. aeruginosa	**Burns**	**50**	**22**	**30.137**
E. coli	**Stool**	**50**	**18**	**24.657**
E. cloacae			**15**	**20.548**
P. mirabilis	**Urine**	**50**	**18**	**24.657**
Total	**All isolates 48.667 %**	**150**	**73**	**99.999**

This table showed the percent of all isolated was 48.667, as well as *Ps. aeruginosa* isolated from burn, *E. coli* from Stool, *E. cloacae* from Urine and *P. mirabilis* from Urine were 30.137, 24.657, 20.548 and 24.657 respectively.

Identification of isolates:.

Table:2. Identification of pathogenic bacteria by ten biochemical tests

Bacterial	Gram stain	Indol	Urease	Oxidase	Catalase	Citrate	MR	VP	H2S	Gas
Ps. aeruginosa*	-	-	-	+	+	+	-	-	-	-
E. coli*	-	+	-	-	+	-	+	-	-	+
E. cloacae**	-	-	-	-	+	+	-	+	-	+
P. mirabilis***	-	-	+	-	+	+	+	+	+	+

This table explained the results of biochemical tests for identified isolates after growing on three plates, were Nutrient agar, Mac Conkey's agar and Blood agar. These bacteria were identified by biochemical tests according to the Society of American Bacteriologists (Pelczar *et al*., 1957). The (+) refer to positive and (–) to negtive (*Cheesbrough., 2006 ; **Otoikhian & Tanimowo 2016 ; ***Stiles & LAI., 1981 and ****Holt *et al*., 1994)

Identification of antibiotics sensitivity and resistant for bacterial samples:.

Antibiotic susceptibility test:

Antibiotic sensitivity tests for these bacteria were carried out by disc diffusion technique on Muller Hinton agar plates (Bauer *et al*., 1966).

Statistical analysis:

The statistical analysis was performed with the Statistical Package for Social Sciences (SPSS) version 20 for calculate Mean, Medium Std. Deviation and Std. Error of Mean as well as the significance between antibiotics according to One-Way ANOVA by descriptive exclude cases analysis by analysis with LSD at 95% confidence and significant level (P=0.05)

III. Results

One hundred and fifty Samples have been collected, through the period from January/2016 to June/2016. This for isolation and identification of pathogenic gram negative bacteria. The samples were involve 50 burns samples for isolate *Pseudomonas aeruginosa*, 50 stool samples for isolate *Escherichia coli* and 50 urine samples for isolate *Enterobacter cloacae* and *Proteus mirabilis***.**

Figure:1. The inhibition zones formed by different types of antibiotics

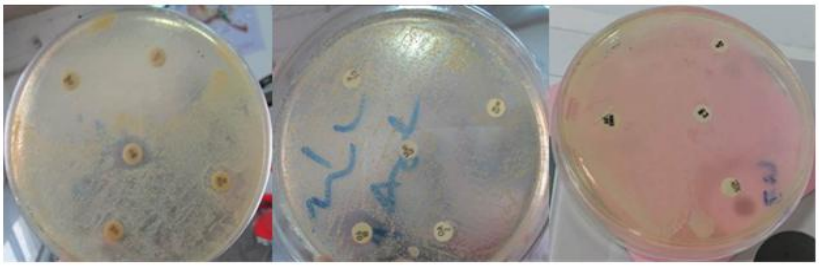


Table:3. Show the replicates of inhibition zones formed by different types of antibiotics against *Ps. Aeruginosa*

	Replicates of Inhibition zones measured by (mm)												
Replicates/*	AX 15 µg	AK 30 µg	TMP 10 µg	ATM 30 µg	SMZ 25 µg	PY 100 µg	NV 30 µg	E 15 µg	R 40 µg	P 10 µg	OA 2 µg	B 10 µg	CC 5 µg
1	7	6	10	12	0	0	10	5	4	1	0	18	10
2	5	10	9	0	4	3	7	6	0	6	2	0	0
3	10	5	7	6	0	0	6	9	0	2	0	5	6
4	5	0	13	0	3	5	8	15	8	5	0	11	3
5	12	0	5	5	0	0	15	8	5	4	3	5	0
6	7	18	12	6	5	5	7	6	5	0	4	0	4
7	4	5	7	0	2	7	13	3	2	6	4	10	4
8	5	2	11	12	3	9	5	12	5	3	8	4	1
9	10	4	15	10	10	6	14	9	9	6	6	6	5
10	8	5	5	2	7	5	6	7	5	7	5	5	0
11	9	9	15	8	5	7	12	9	7	4	6	7	6
12	17	7	15	0	0	5	9	0	3	0	8	6	8
13	10	8	7	16	13	10	10	18	10	10	4	8	4
14	0	0	8	6	8	7	8	7	0	8	9	5	1
15	0	10	19	0	7	8	7	11	7	3	3	7	3
Mean	7.266	5.933	10.533	5.533	4.466	5.134	9.133	8.333	4.667	4.333	4.133	6.466	3.667
Median	7.000	5.000	10.000	6.000	4.000	5.000	8.000	8.000	5.000	4.000	4.000	6.000	4.000
Std. Deviation	4.431	4.787	4.206	5.249	3.961	3.181	3.113	4.498	3.221	2.919	2.924	4.374	3.015
Std. Error of Mean	1.144	1.236	1.086	1.355	1.022	0.821	0.803	1.161	0.821	0.753	0.756	1.129	0.779

This table explained the Mean, Median, Std. deviation and Std. error for replicates of inhibition zones that measured by (mm) for each antibiotics against *Ps. aeruginosa* bacteria. And showed the (TMP 10) antibiotic has high activity (10.533 mm) while the (CC 5) antibiotic has low activity (3.667 mm) against *Ps. aeruginosa* bacteria by mean measured.

*AX 15	Amoxicillin15 µg	*E 15	Erythromycin15 µg
*AK 30	Amikacin30 µg	*R 40	Rifaximin40 µg
*TMP 10	Trimethoprim10 µg	*P 10	Penicillin-G10 µg
*ATM 30	Aztreonam30 µg	*OA 2	Oxolinic acid2 µg
*SMZ 25	Sulfamethoxazole25 µg	*B 10	Bacitracin10 µg
*PY 100	Carbnicillin100 µg	*CC 5	Clindamycin5 µg
*NV 30	Novobiocin30 µg		

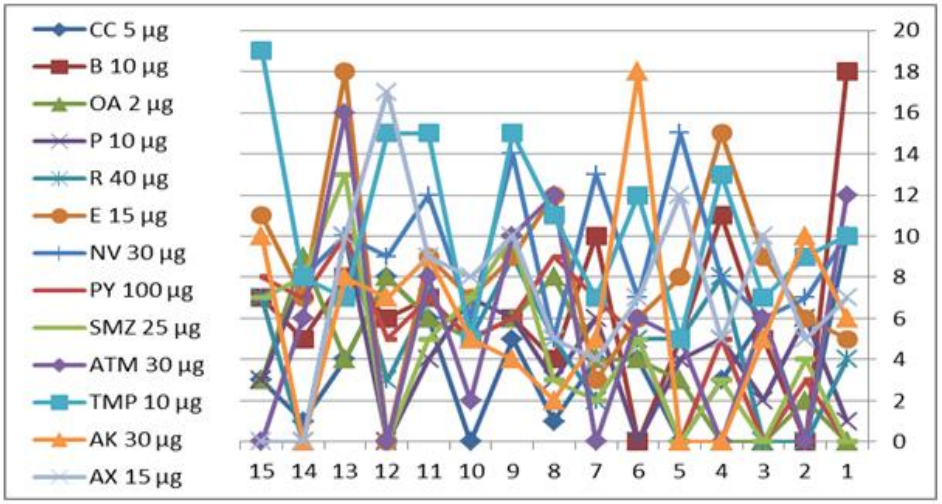
Table:4. Show the significantly study according to LSD system for different types of antibiotics against *Ps. Aeruginosa*

Significantly study according to LSD at P-Value = 0.05														
Y side / X side (antibiotics)		AX15 µg	AK30 µg	TMP10 µg	ATM30 µg	SMZ25 µg	PY100 µg	NV30 µg	E 15 µg	R 40 µg	P 10 µg	OA 2 µg	B 10 µg	CC 5 µg
AX	Sig.		0.352	0.002*	0.227	0.052	0.137	0.193	0.457	0.071	0.042*	0.031*	0.576	0.013*
15 µg	M.D. **		-1.333	4.601	-1.733	-2.801	-2.133	1.866	1.066	-2.601	-2.933	-3.133	-0.801	-3.601
AK	Sig.	0.352		0.002*	0.780	0.306	0.576	0.026*	0.095	0.377	0.264	0.210	0.710	0.115
30 µg	M.D. **	1.333		4.601	-0.401	-1.466	-0.801	3.201	2.401	-1.266	-1.601	-1.801	0.533	-2.266
TMP	Sig.	0.023*	0.002*		0.001*	0.001*	0.001*	0.329	0.126	0.001*	0.001*	0.001*	0.005*	0.001*
10 µg	M.D. **	-3.266	-4.601		-5.001	-6.066	-5.401	-1.401	-2.201	-5.866	-6.201	-6.401	-4.066	-6.866
ATM	Sig.	0.227	0.780	0.001*		0.457	0.780	0.013*	0.052	0.545	0.402	0.329	0.515	0.193
30 µg	M.D. **	1.733	0.401	5.001		-1.066	-0.401	3.601	2.801	-0.866	-1.201	-1.401	0.933	-1.866
SMZ	Sig.	0.052	0.306	0.001*	0.457		0.642	0.001*	0.007*	0.889	0.926	0.816	0.163	0.576
25 µg	M.D. **	2.801	1.466	6.066	1.066		0.666	4.666	3.866	0.201	-0.133	-0.333	2.001	-0.801
PY	Sig.	0.137	0.576	0.001*	0.780	0.642		0.006*	0.026*	0.744	0.576	0.485	0.352	0.306
100 µg	M.D. **	2.133	0.801	5.401	0.401	-0.666		4.001	3.201	-0.466	-0.801	-1.001	1.333	-1.466
NV	Sig.	0.193	0.026*	0.329	0.013*	0.001*	0.006*		0.576	0.002*	0.001*	0.001*	0.064	0.001*
30 µg	M.D. **	-1.866	-3.201	1.401	-3.601	-4.666	-4.001		-0.801	-4.466	-4.801	-5.001	-2.666	-5.466
E	Sig.	0.457	0.095	0.126	0.052	0.007*	0.026*	0.576		0.011*	0.006*	0.004*	0.193	0.001*
15 µg	M.D. **	-1.066	-2.401	2.201	-2.801	-3.866	-3.201	0.801		-3.666	4.001	-4.201	-1.866	-4.666
R	Sig.	0.071	0.377	0.001*	0.545	0.889	0.744	0.002*	0.011*		0.816	0.710	0.211	0.485
40 µg	M.D. **	2.601	1.266	5.866	0.866	-0.201	0.466	4.466	3.666		-0.333	-0.533	1.801	-1.001
P	Sig.	0.042*	0.264	0.001*	0.402	0.926	0.576	0.001*	0.006*	0.816		0.889	0.137	0.642
10 µg	M.D. **	2.933	1.601	6.201	1.201	0.133	0.801	4.801	4.001	0.333		-0.201	2.133	-0.666
OA	Sig.	0.031*	0.210	0.001*	0.329	0.816	0.485	0.001*	0.004*	0.710	0.889		0.104	0.744
2 µg	M.D. **	3.133	1.801	6.401	1.401	0.333	1.001	5.001	4.201	0.533	0.201		2.333	-0.466
B	Sig.	0.576	0.710	0.005*	0.515	0.163	0.352	0.064	0.193	0.211	0.137	0.104		0.052
10 µg	M.D. **	0.801	-0.533	4.066	-0.933	-2.001	-1.333	2.666	1.866	-1.801	-2.133	-2.333		-2.801
CC	Sig.	0.013*	0.115	0.001*	0.193	0.576	0.306	0.001*	0.001*	0.485	0.642	0.744	0.052	
5 µg	M.D. **	3.601	2.266	6.866	1.866	0.801	1.466	5.466	4.666	1.001	0.666	0.466	2.801	

***The mean difference is significant at the P-Value = 0.05 level.**
****Mean difference.**

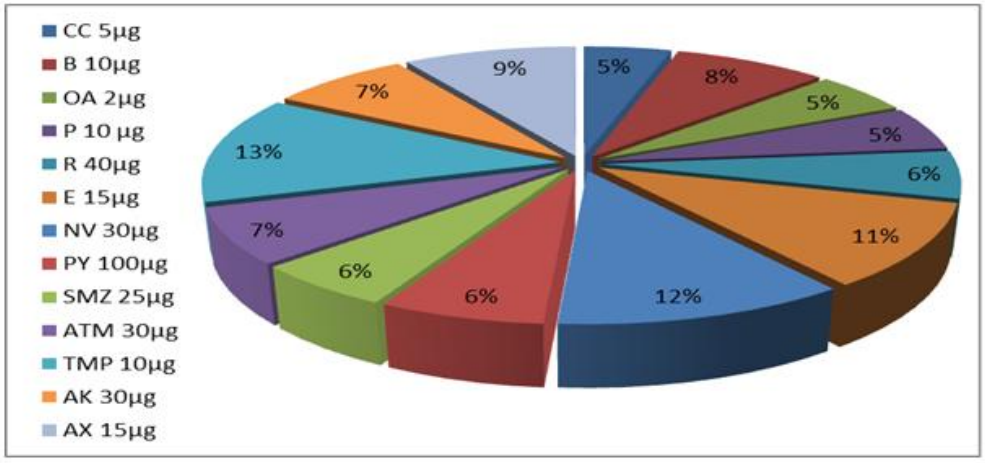
This table explained the significantly differences between different types of antibiotics when study their activities against *Ps. aeruginosa* by statistical analysis depended on LSD system. Also showed presences of significant differences between these antibiotics, and this explained through the star that found at p-valve equal or less than 0.05 level However, not all antibiotics have significant differences when comparison with each other, some of them have no significant differences, and this showed through the star that not found at p-valve at 0.05 level.The comparisons involved two types, first the X side (antibiotics) comparison with Y side (antibiotics) and the second involved comparison the Y side (antibiotics) with X side (antibiotics).

Figure:2. Show the modes of action of different types of antibiotics against ***Ps. Aeruginosa***



This figure explained the antibiotics types have different modes of actions, this mean each antibiotic showed inhibition zone with different size against same bacteria with several replicates (15 replicates).

Figure:3. Show the percent of action of different types of antibiotics against ***Ps. aeruginosa***



This figure showed the action percentages of different antibiotic types; also explained the TMP 10 antibiotics has high percent equal to 13% while the CC 5 antibiotic, P 10 antibiotic and OA 2 antibiotic all have low percent equal to 5% from all antibiotics activities against *Ps. Aeruginoa*

Table:5. Show the replicates of inhibition zones formed by different types of antibiotics against *E.coli*

Replicates of Inhibition zones measured by (mm)													
Replicates/*	AX 15 µg	AK 30 µg	TMP 10 µg	ATM 30 µg	SMZ 25 µg	PY 100 µg	NV 30 µg	E 15 µg	R 40 µg	P 10 µg	OA 2 µg	B 10 µg	CC 5 µg
1	7	10	12	9	7	1	0	1	5	0	2	0	6
2	10	9	7	8	9	2	5	1	0	5	5	2	10
3	8	15	13	7	6	1	3	5	10	10	0	6	0
4	0	10	0	6	5	3	9	0	0	1	9	0	2
5	8	11	11	10	12	5	10	1	0	0	5	0	3
6	12	9	10	9	8	5	5	4	3	4	4	4	4
7	9	19	8	7	11	4	5	0	6	6	11	3	0
8	0	7	10	6	6	5	6	8	0	0	0	5	8
9	13	10	6	9	5	7	7	12	4	5	9	2	2
10	7	8	12	4	10	5	5	6	2	3	6	6	8
11	9	12	11	11	8	7	7	6	6	7	0	6	6
12	10	10	16	13	12	0	4	0	0	10	7	6	0
13	8	17	15	0	6	0	12	12	2	0	10	0	12
14	10	14	10	16	8	14	9	12	4	0	0	10	8
15	11	17	14	22	0	14	7	0	8	10	12	8	16
Mean	8.134	11.867	10.333	9.134	7.534	4.867	6.267	4.534	3.334	4.067	5.334	3.867	5.667
Median	9.000	11.000	11.000	9.000	8.000	5.000	6.000	4.000	3.000	4.000	5.000	4.000	6.000
Std. Deviation	3.719	3.661	3.995	5.123	3.137	4.356	2.988	4.657	3.199	3.899	4.271	3.182	4.746
Std. Error of Mean	0.961	0.955	1.032	1.331	0.809	1.125	0.771	1.203	0.827	1.007	1.103	0.822	1.226

This table explained the Mean, Median, Std. deviation and Std. error for replicates of inhibition zones that measured by (mm) for each antibiotics against *E. coli* bacteria. And showed the (AK 30) antibiotic has high activity (11.867 mm) while the (B 10) antibiotic has low activity (3.867mm) against *E. coli* bacteria by mean measured.

***AX 15**	**Amoxicillin15 µg**	***E 15**	**Erythromycin15 µg**
***AK 30**	**Amikacin30 µg**	***R 40**	**Rifaximin40 µg**
***TMP 10**	**Trimethoprim10 µg**	***P 10**	**Penicillin-G10 µg**
***ATM 30**	**Aztreonam30 µg**	***OA 2**	**Oxolinic acid2 µg**
***SMZ 25**	**Sulfamethoxazole25 µg**	***B 10**	**Bacitracin10 µg**
***PY 100**	**Carbnicillin100 µg**	***CC 5**	**Clindamycin5 µg**
***NV 30**	**Novobiocin30 µg**		

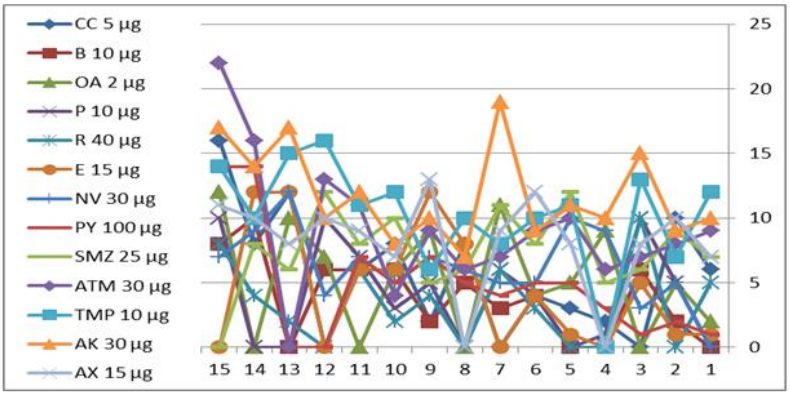
Table:6. Show the significantly study according to LSD system for different types of antibiotics against *E. coli*

Significantly study according to LSD at P-Value = 0.05														
Y side / X side (antibiotics)		AX15 µg	AK30 µg	TMP10 µg	ATM30 µg	SMZ25 µg	PY100 µg	NV30 µg	E 15 µg	R 40 µg	P 10 µg	OA 2 µg	B 10 µg	CC 5 µg
AX	Sig.		0.011*	0.131	1.001	0.680	0.026*	0.200	0.014*	0.001*	0.006*	0.055	0.004*	-0.091
15 µg	M.D. **		3.733	2.201	0.492	-0.601	-3.266	-1.866	-3.601	-4.801	-4.066	-2.801	-4.266	2.466
AK	Sig.	0.011*		0.292	0.061	0.003*	0.011*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*
30 µg	M.D. **	-3.733		-1.533	-2.733	-4.333	-7.00	-5.601	-7.333	-8.533	-7.801	-6.533	-8.001	-6.201
TMP	Sig.	0.131	0.29		0.410	0.055	0.011*	0.006*	0.001*	0.001*	0.001*	0.001*	0.001*	0.002*
10 µg	M.D. **	-2.201	1.533		-1.201	-2.801	-5.466	-4.066	-5.801	-7.001	-6.266	-5.001	-6.466	-4.666
ATM	Sig.	0.492	0.06	0.410		0.272	0.004*	0.051*	0.002*	0.001*	0.001*	0.011*	0.001*	0.018*
30 µg	M.D. **	-1.001	2.733	1.201		-1.601	-4.266	-2.866	-4.601	-5.801	-5.066	-3.801	-5.266	-3.466
SMZ	Sig.	0.681	0.003*	0.055	0.272		0.068	0.384	0.014*	0.004*	0.018	0.131	0.012*	0.200
25 µg	M.D. **	0.601	4.33	2.801	1.601		-2.666	-1.266	-3.001	-4.201	-3.466	-2.201	-3.666	-1.866
PY	Sig.	0.026*	0.001*	0.001*	0.004*	0.068		0.336	0.819	0.292	0.582	0.748	0.492	0.582
100 µg	M.D. **	3.601	7.001	5.466	4.266	2.666		1.401	-0.333	-1.533	-0.801	-0.466	-1.001	1.801
NV	Sig.	0.201	0.001*	0.006*	0.051*	0.384	0.336		0.234	0.045*	0.131	0.521	0.101	0.680
30 µg	M.D. **	1.866	5.601	4.066	2.866	1.266	-1.401		-1.733	-2.933	-2.201	-0.933	-2.401	-0.601
E	Sig.	0.014*	0.001*	0.001*	0.002*	0.014*	0.819	0.234		0.410	0.748	0.582	0.647	0.436
15 µg	M.D. **	3.601	7.333	5.801	4.601	3.001	0.333	-1.733		-1.201	-0.466	0.801	0.666	-1.133
R	Sig.	0.001*	0.001*	0.001*	0.001*	0.004*	0.292	0.045*	0.410		0.614	0.170	0.714	0.110
40 µg	M.D. **	4.801	8.533	8.533	5.801	4.201	1.533	2.933	1.201		-0.733	2.001	-0.533	2.333
P	Sig.	0.006*	0.001*	0.001*	0.001*	0.018	0.582	0.131	0.748	0.614		0.384	0.891	0.272
10 µg	M.D. **	4.066	7.801	6.266	5.066	3.466*	0.801	2.201	0.466	-0.733		1.266	-0.201	1.601
OA	Sig.	0.055	0.001*	0.001*	0.011*	0.131	0.748	0.521	0.582	0.170	0.384		0.314	0.819
2 µg	M.D. **	2.801	6.533	5.001	3.801	2.201	-0.466	0.933	-0.801	-2.001	-1.266		-1.466	0.333
B	Sig.	0.004*	0.001*	0.001*	0.001*	0.012*	0.492	0.101	0.647	0.714	0.891	0.314		0.217
10 µg	M.D. **	4.266	8.001	6.466	5.266	3.666	1.001	2.401	0.666	-0.533	0.201	1.466		1.801
CC	Sig.	0.091	0.001*	0.002*	0.018*	0.200	0.582	0.680	0.436	0.110	0.272	0.819	0.217	
5 µg	M.D. **	2.466	6.201	4.666	3.466	1.866	-1.801	0.601	-1.133	-2.333	-1.601	-0.333	-1.801	

***The mean difference is significant at the P-Value = 0.05 level.**
****Mean difference.**

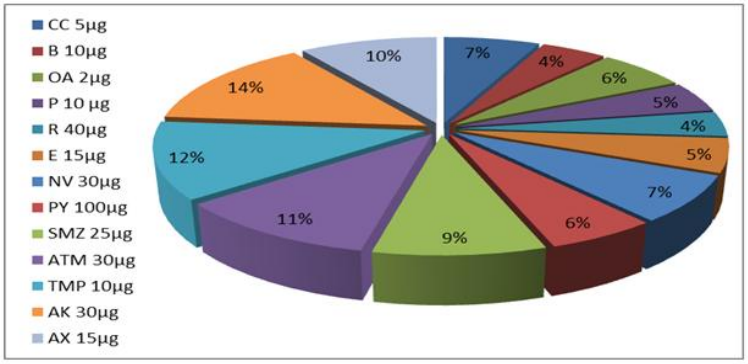
This table explained the significantly differences between different types of antibiotics when study their activities against *E. coli* by statistical analysis depended on LSD system. Also showed presences of significant differences between these antibiotics, and this explained through the star that found at p-valve equal or less than 0.05 level.However, not all antibiotics have significant differences when comparison with each other, some of them have no significant differences, and this showed through the star that not found at p-valve at 0.05 level. The comparisons involved two types, first the X side (antibiotics) comparison with Y side (antibiotics) and the second involved comparison the Y side (antibiotics) with X side (antibiotics).

Figure:4. Show the modes of action of different types of antibiotics against *E. coli*



This figure explained the antibiotics types have different modes of actions, this mean each antibiotic showed inhibition zone with different size against same bacteria with several replicates (15 replicates).

Figure:5. Show the percent of action of different types of antibiotics against *E. coli*



This figure showed the action percentages of different antibiotic types; also explained the AK 30 antibiotics has high percent equal to 14% while the B10 antibiotic and R 40 antibiotic both have low percent equal to 4% from all antibiotics activities against *E. coli*.

Table:7. Show the replicates of inhibition zones formed by different types of antibiotics against *Enterobacter cloacae*

Replicates of Inhibition zones measured by (mm)													
Replicates/*	AX 15 µg	AK 30 µg	TMP 10 µg	ATM 30 µg	SMZ 25 µg	PY 100 µg	NV 30 µg	E 15 µg	R 40 µg	P 10 µg	OA 2 µg	B 10 µg	CC 5 µg
1	6	6	13	15	5	0	5	10	9	6	5	0	0
2	7	3	7	13	7	7	9	8	6	5	6	5	7
3	3	0	9	16	10	0	4	9	4	10	13	0	3
4	9	8	12	20	9	0	7	7	7	4	3	3	0
5	8	7	11	18	4	5	3	6	5	6	4	9	3
6	9	7	9	20	0	6	8	10	10	7	8	5	6
7	14	14	10	15	10	5	16	15	15	0	1	5	3
8	0	0	8	25	4	1	6	8	18	4	6	3	9
9	15	8	10	17	5	11	0	14	13	12	15	2	2
10	8	6	8	23	9	7	10	7	6	9	10	0	10
11	12	9	6	22	5	8	10	12	8	9	5	7	8
12	5	7	9	30	7	3	15	10	12	2	15	11	0
13	10	11	0	14	6	16	6	6	18	8	9	1	16
14	19	8	16	23	11	0	13	18	5	5	4	2	11
15	15	10	5	21	3	5	7	14	6	4	8	11	5
Mean	9.333	6.934	8.867	19.466	6.333	4.934	7.933	10.267	9.466	6.067	7.466	4.253	5.534
Median	9.000	7.000	9.000	20.000	6.000	5.000	7.000	10.000	8.000	6.000	6.000	3.000	5.000
Std. Deviation	5.023	3.751	3.701	4.688	3.062	4.589	4.399	3.614	4.703	3.788	4.274	3.788	4.657
Std. Error of Mean	1.297	0.968	0.955	1.211	0.7908	1.185	1.135	0.933	1.214	0.819	1.104	0.978	1.203

This table explained the Mean, Median, Std. deviation and Std. error for replicates of inhibition zones that measured by (mm) for each antibiotics against *Enterobacter cloacae* bacteria. And showed the (ATM 30) antibiotic has high activity (19.466 mm) while the (PY 100) antibiotic has low activity (4.934 mm) against *Enterobacter cloacae* bacteria by mean measured.

*AX 15	Amoxicillin15 µg	*E 15	Erythromycin15 µg
*AK 30	Amikacin30 µg	*R 40	Rifaximin40 µg
*TMP 10	Trimethoprim10 µg	*P 10	Penicillin-G10 µg
*ATM 30	Aztreonam30 µg	*OA 2	Oxolinic acid2 µg
*SMZ 25	Sulfamethoxazole25 µg	*B 10	Bacitracin10 µg
*PY 100	Carbnicillin100 µg	*CC 5	Clindamycin5 µg
*NV 30	Novobiocin30 µg		

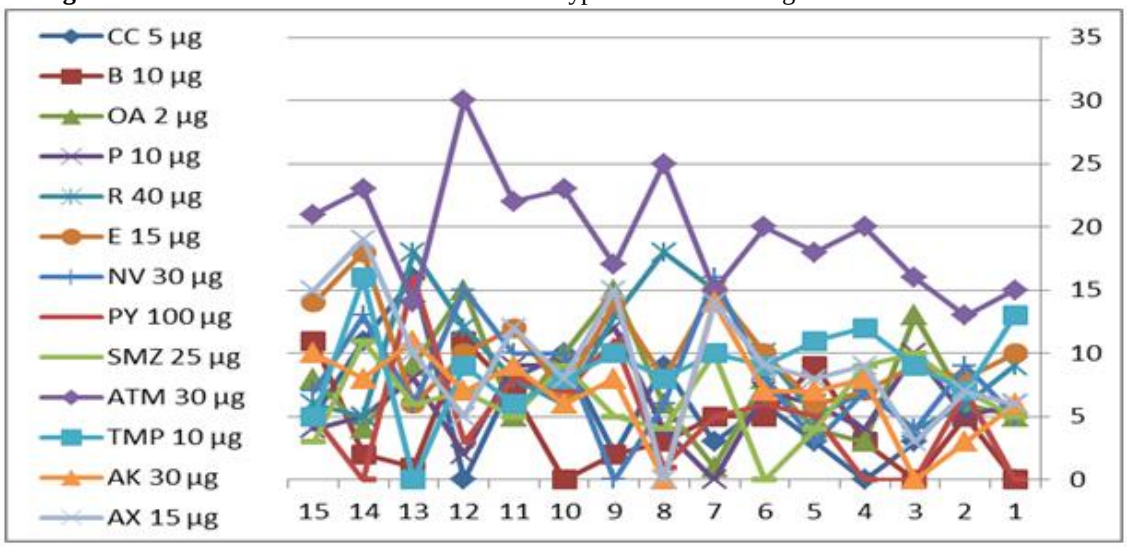
Table:8. Show the significantly study according to LSD system for different types of antibiotics against *Enterobacter cloacae*

Significantly study according to LSD at P-Value = 0.05														
Y side / X side (antibiotics)		AX15 µg	AK30 µg	TMP10 µg	ATM30 µg	SMZ2 5 µg	PY100 µg	NV30 µg	E 15 µg	R 40 µg	P 10 µg	OA 2 µg	B 10 µg	CC 5 µg
AX 15 µg	Sig.		0.115	0.759	0.001*	0.049*	0.004*	0.357	0.539	0.930	0.033*	0.220	0.001*	0.013*
	M.D. **		-2.401	-0.466	-10.133	-3.001	-4.401	-1.401	0.933	0.133	-3.266	-1.866	-5.066	-3.801
AK 30 µg	Sig.	0.115		0.204	0.001*	0.693	0.189	0.357	0.029*	0.097	0.568	0.726	0.080	0.357
	M.D. **	2.401		1.933	-12.533	-0.601	-2.001	1.401	3.333	2.533	-0.866	0.533	-2.666	-1.401
TMP 10 µg	Sig.	0.759	0.204		0.001*	0.097	0.011*	0.539	0.357	0.693	0.067	0.357	0.003*	0.029*
	M.D. **	0.466	-1.933		10.600	-2.533	-3.933	-0.933	1.401	0.601	-2.801	-1.401	-4.601	-3.333
ATM 30 µg	Sig.	0.001*	0.001*	0.001*		0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*
	M.D. **	10.133	12.533	-10.600		-13.133	-14.533	-11.533	-9.201	-10.001	-13.401	-12.001	-15.201	-13.933
SMZ 25 µg	Sig.	0.049*	0.693	0.097	0.001*		0.357	0.293	0.011*	0.041*	0.861	0.456	0.175	0.599
	M.D. **	3.001	0.601	2.533	13.133		-1.401	1.601	3.933	3.133	0.266	1.133	-2.066	-0.801
PY 100 µg	Sig.	0.004*	0.189	0.011*	0.001*	0.357		0.049*	0.011*	0.003*	0.456	0.097	0.661	0.693
	M.D. **	4.401	2.001	3.933	14.533	1.401		3.001	5.333	4.533	1.133	2.533	-0.666	0.601
NV 30 µg	Sig.	0.357	0.357	0.539	0.001*	0.293	0.049*		0.126	0.313	0.220	0.759	0.017*	0.115
	M.D. **	1.401	1.401	0.933	11.533	-1.601	-3.001		2.333	1.533	-1.866	-0.466	-3.666	-2.401
E 15 µg	Sig.	0.539	0.029*	0.357	0.001*	0.011*	0.011*	0.126		0.599	0.006*	0.067	0.001*	0.002
	M.D. **	-0.933	-3.333	-1.401	9.201	-3.933	-5.333	-2.333		-0.801	-4.201	-2.801	-6.001	-4.733
R 40 µg	Sig.	0.930	0.097	0.693	0.001*	0.041*	0.003*	0.313	0.599		0.026*	0.189	0.001*	0.011*
	M.D. **	-0.133	-2.533	-0.601	10.001	-3.133	-4.533	-1.533	0.801		-3.401	-2.001	-5.201	-3.933
P 10 µg	Sig.	0.033*	0.568	0.067	0.001*	0.861	0.456	0.220	0.006*	0.026*		0.357	0.237	0.726
	M.D. **	3.266	0.866	2.801	13.401	0.266	-1.133	1.866	4.201	3.401		1.401	-1.801	-0.533
OA 2 µg	Sig.	0.220	0.726	0.357	0.001*	0.456	0.097	0.759	0.067	0.189	0.357		0.036*	0.204
	M.D. **	1.866	-0.533	1.401	12.001	-1.133	-2.533	0.466	2.801	2.001	-1.401		-3.201	-1.933
B 10 µg	Sig.	0.001*	0.080	0.003*	0.001*	0.175	0.661	0.017*	0.001*	0.001*	0.237	0.036*		0.405
	M.D. **	5.066	2.666	4.601	15.201	2.066	0.666	3.666	6.001	5.201	1.801	3.201		1.266
CC 5 µg	Sig.	0.013*	0.357	0.029*	0.001*	0.599	0.693	0.115	0.002	0.011*	0.726	0.204	0.405	
	M.D. **	3.801	1.401	3.333	13.933	0.801	-0.601	2.401	4.733	3.933	0.533	1.933	-1.266	

***The mean difference is significant at the P-Value = 0.05 level.**
****Mean difference.**

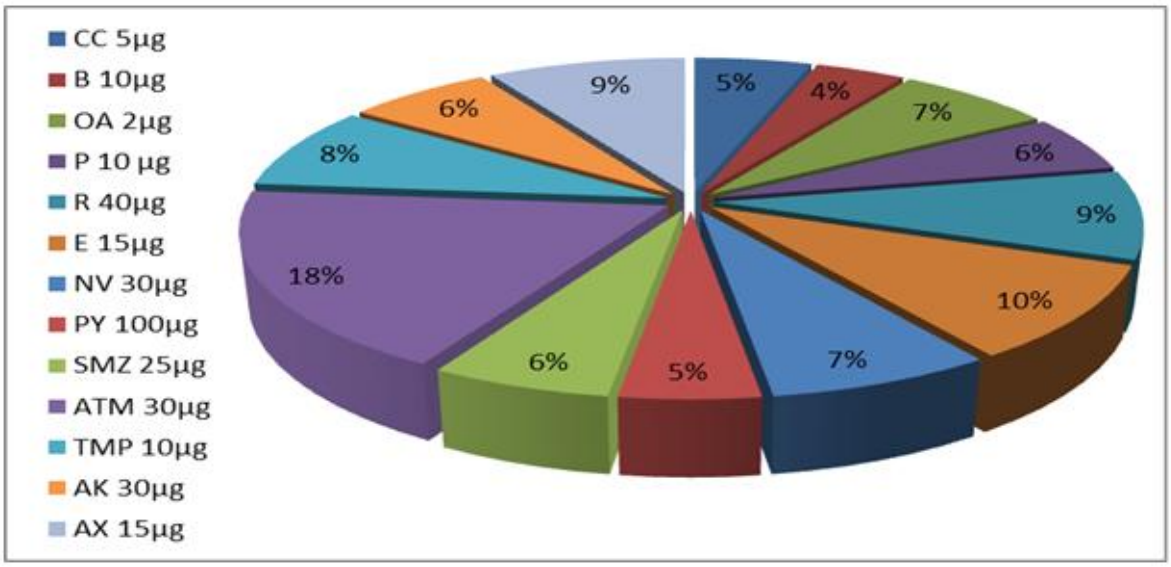
This table explained the significantly differences between different types of antibiotics when study their activities against *E. cloacae* by statistical analysis depended on LSD system.Also showed presences of significant differences between these antibiotics, and this explained through the star that found at p-valve equal or less than 0.05 level.However, not all antibiotics have significant differences when comparison with each other, some of them have no significant differences, and this showed through the star that not found at p-valve at 0.05 level.The comparisons involved two types, first the X side (antibiotics) comparison with Y side (antibiotics) and the second involved comparison the Y side (antibiotics) with X side (antibiotics).

Figure:6. Show the modes of action of different types of antibiotics against ***Enterobacter cloacae***



This figure explained the antibiotics types have different modes of actions, this mean each antibiotic showed inhibition zone with different size against same bacteria with several replicates (15 replicates).

Figure:7. Show the percent of action of different types of antibiotics against ***Enterobacter cloacae***



This figure showed the action percentages of different antibiotic types; also explained the ATM 30 antibiotics has high percent equal to 18% while the B10 antibiotic has low percent equal to 4% from all antibiotics activities against *Enterobacter cloacae*.

Table:9. Show the replicates of inhibition zones formed by different types of antibiotics against *Proteus mirabilis*

Replicates of Inhibition zones measured by (mm)													
Replicates/*	AX 15 µg	AK 30 µg	TMP 10 µg	ATM 30 µg	SMZ 25 µg	PY 100 µg	NV 30 µg	E 15 µg	R 40 µg	P 10 µg	OA 2 µg	B 10 µg	CC 5 µg
1	6	8	0	8	0	6	11	8	12	8	16	11	8
2	0	5	5	7	9	12	9	9	7	5	0	6	7
3	10	6	6	15	13	5	20	7	20	8	12	5	6
4	6	8	0	3	12	6	14	8	9	5	6	0	4
5	5	6	8	0	6	5	19	15	7	0	5	5	10
6	7	7	6	11	8	9	13	11	10	7	7	7	7
7	4	8	0	10	0	5	14	9	13	3	4	5	5
8	10	12	12	12	16	0	6	11	8	7	12	9	8
9	5	14	5	16	6	10	16	16	9	0	9	3	9
10	9	6	7	14	14	7	10	18	14	5	8	0	10
11	9	10	8	10	10	9	16	11	13	7	11	11	12
12	16	15	6	0	0	8	7	15	15	0	5	5	8
13	2	5	10	7	15	2	9	9	13	8	7	17	13
14	0	20	16	14	6	0	12	7	11	6	10	8	6
15	8	0	0	16	7	20	15	0	9	5	0	14	11
Mean	6.467	8.667	5.933	9.534	8.134	6.934	12.734	10.2667	11.334	4.934	7.467	7.067	8.266
Median	6.000	8.000	6.000	10.000	8.000	6.000	13.000	9.000	11.000	5.000	7.000	6.000	8.000
Std. Deviation	4.189	4.909	4.683	5.343	5.344	4.994	4.132	4.479	3.499	2.915	4.422	4.758	2.575
Std. Error of Mean	1.082	1.167	1.109	1.384	1.379	1.289	1.067	1.157	0.904	0.753	1.142	1.129	0.341

This table explained the Mean, Median, Std. deviation and Std. error for replicates of inhibition zones that measured by (mm) for each antibiotics against *Proteus mirabilis* bacteria. And showed the (NV 30) antibiotic has high activity (12.734 mm) while the (P 10) antibiotic has low activity (4.934 mm) against *Proteus mirabilis* bacteria by mean measured.

*AX 15	Amoxicillin15 µg	*E 15	Erythromycin15 µg
*AK 30	Amikacin30 µg	*R 40	Rifaximin40 µg
*TMP 10	Trimethoprim10 µg	*P 10	Penicillin-G10 µg
*ATM 30	Aztreonam30 µg	*OA 2	Oxolinic acid2 µg
*SMZ 25	Sulfamethoxazole25 µg	*B 10	Bacitracin10 µg
*PY 100	Carbnicillin100 µg	*CC 5	Clindamycin5 µg
*NV 30	Novobiocin30 µg		

Table:10. Show the significantly study according to LSD system for different types of antibiotics against *Proteus mirabilis*

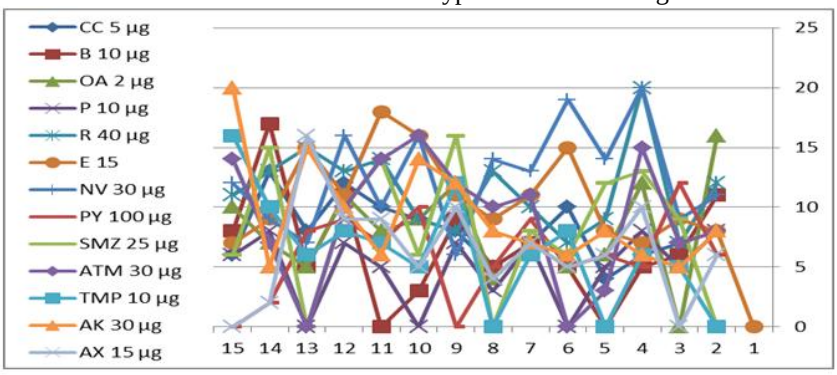
Significantly study according to LSD at P-Value = 0.05														
Y side / X side (antibiotics)		AX15 µg	AK30 µg	TMP10 µg	ATM30 µg	SMZ25 µg	PY100 µg	NV30 µg	E 15 µg	R 40 µg	P 10 µg	OA 2 µg	B 10 µg	CC 5 µg
AX 15 µg	Sig.		0.173	0.740	0.058	0.301	0.772	0.001*	0.019*	0.003*	0.341	0.535	0.709	0.264
	M.D. **		2.200	-0.533	3.066	1.666	0.466	6.266	3.801	4.866	-1.533	1.001	0.600	1.801
AK 30 µg	Sig.	0.173		0.091	0.590	0.740	0.282	0.012*	0.321	0.099	0.021*	0.456	0.321	0.803
	M.D. **	-2.200		-2.733	0.866	-0.533	-1.733	4.066	1.601	2.666	-3.733	-1.201	-1.601	-0.401
TMP 10 µg	Sig.	0.740	0.091		0.026*	0.173	0.535	0.001*	0.007*	0.001*	0.535	0.341	0.482	0.148
	M.D. **	0.533	2.733		3.601	2.201	1.001	6.801	4.333	5.401	-1.001	1.533	1.133	2.333
ATM 30 µg	Sig.	0.058	0.590	0.026*		0.385	0.107	0.048*	0.649	0.264	0.004*	0.201	0.127	0.432
	M.D. **	-3.066	-0.866	-3.601		-1.401	-2.601	3.201	0.733	1.801	-4.601	-2.066	-2.466	-1.266
SMZ 25 µg	Sig.	0.301	0.740	0.173	0.385		0.456	0.004*	0.186	0.048*	0.048*	0.679	0.508	0.934
	M.D. **	-1.666	0.533	-2.201	1.401		-1.201	4.601	2.133	3.201	-3.201	-0.666	-1.066	0.133
PY 100 µg	Sig.	0.772	0.282	0.535	0.107	0.456		0.001*	0.041*	0.007*	0.215	0.741	0.934	0.408
	M.D. **	-0.466	1.733	-1.001	2.601	1.201		5.801	3.333	4.401	-2.001	0.533	0.133	1.333
NV 30 µg	Sig.	0.001*	0.012*	0.001*	0.048*	0.004*	0.001*		0.127	0.385	0.001*	0.001*	0.001*	0.006*
	M.D. **	-6.266	-4.066	-6.801	-3.201	-4.601	-5.801		-2.466	-1.401	-7.801	-5.266	-5.666	-4.466
E 15 µg	Sig.	0.019*	0.321	0.007*	0.649	0.186	0.041*	0.127		0.508	0.001*	0.083	0.048*	0.215
	M.D. **	-3.801	-1.601	-4.333	-0.733	-2.133	-3.333	2.466		1.066	-5.333	-2.801	-3.201	-2.001
R 40 µg	Sig.	0.003*	0.099	0.001*	0.264	0.048*	0.007*	0.385	0.508		0.001*	0.017*	0.009*	0.058
	M.D. **	-4.866	-2.666	-5.401	-1.801	-3.201	-4.401	1.401	-1.066		-6.401	-3.866	-4.266	-3.066
P 10 µg	Sig.	0.341	0.021*	0.535	0.004*	0.048*	0.215	0.001*	0.001*	0.001*		0.117	0.186	0.014*
	M.D. **	1.533	3.733	1.001	4.601	3.201	2.001	7.801	5.333	6.401		2.535	2.135	3.333
OA 2 µg	Sig.	0.535	0.456	0.341	0.201	0.679	0.741	0.001*	0.083	0.017*	0.117		0.803	0.619
	M.D. **	-1.001	1.201	-1.533	2.066	0.666	-0.533	5.266	2.801	3.866	-2.535		-0.401	0.801
B 10 µg	Sig.	0.709	0.321	0.482	0.127	0.508	0.934	0.001*	0.048*	0.009*	0.186	0.803		0.456
	M.D. **	-0.600	1.601	-1.133	2.466	1.066	-0.133	5.666	3.201	4.266	-2.133	0.401		1.201
CC 5 µg	Sig.	0.264	0.803	0.148	0.432	0.934	0.408	0.006*	0.215	0.058	0.014*	0.619	0.456	
	M.D. **	-1.801	0.401	-2.333	1.266	-0.133	-1.333	4.466	2.001	3.066	-3.333	-0.801	-1.201	

***The mean difference is significant at the P-Value = 0.05 level.**
****Mean difference.**

This table explained the significantly differences between different types of antibiotics when study their activities against *P. mirabilis* by statistical analysis depended on LSD system.
Also showed presences of significant differences between these antibiotics, and this explained through the star that found at p-valve equal or less than 0.05 level.

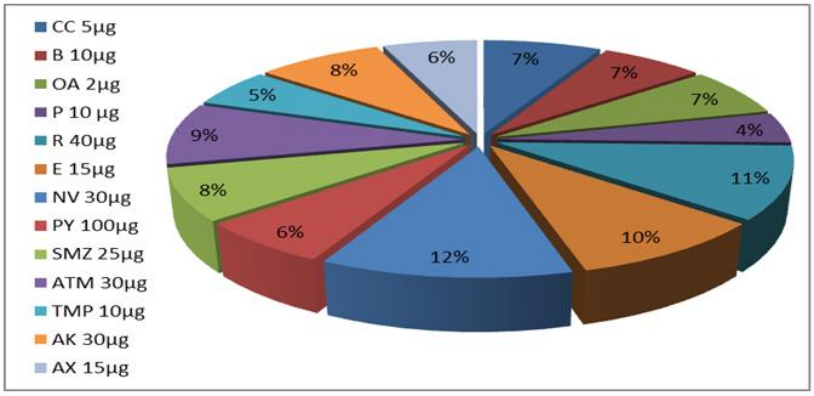
However, not all antibiotics have significant differences when comparison with each other, some of them have no significant differences, and this showed through the star that not found at p-valve at 0.05 level.
The comparisons involved two types, first the X side (antibiotics) comparison with Y side (antibiotics) and the second involved comparison the Y side (antibiotics) with X side (antibiotics).

Figure:8. Show the modes of action of different types of antibiotics against ***Proteus mirabilis***



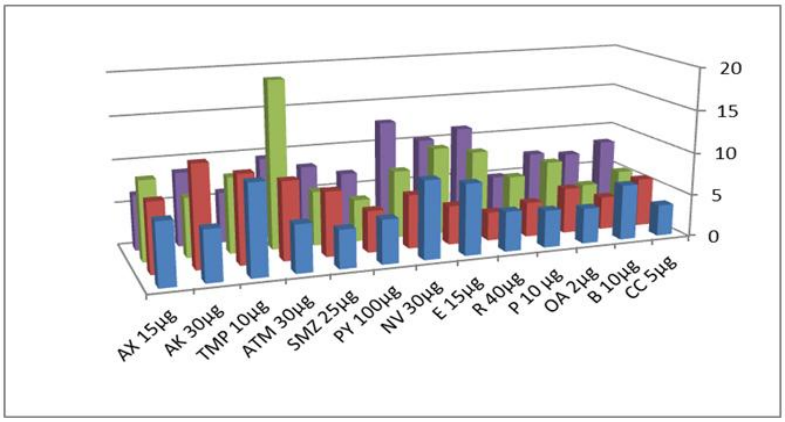
This figure explained the antibiotics types have different modes of actions, this mean each antibiotic showed inhibition zone with different size against same bacteria with several replicates (15 replicates).

Figure:9. Show the percent of action of different types of antibiotics against ***Proteus mirabilis***



This figure showed the action percentages of different antibiotic types; also explained the NV 30 antibiotics has high percent equal to 12% while the P 10 antibiotic has low percent equal to 4% from all antibiotics activities against *Proteus mirabilis*.

Figure:10. Show the comparisons between all antibiotics means against pathogenic gram negative



This figure showed the ATM 30 (Aztreonam30) antibiotic has high activity against in comparison with all other types of antibiotics.

IV. Discussion

In this study showed the (Trimethoprim) antibiotic has high activity against *Ps. aeruginosa* (Table:3 and Figure:3.). These results were agreed with the Kamel *et al* who found the isolate of this bacteria were sensitive to this antibiotic (Kamel *et al*., 2011). While dis agreed with Rosas *et al* who found the isolate of this bacteria were resistant to trimethoprim (Rosas *et al*., 2015). The present study explained the (Clindamycin) antibiotic has low activity against *Pseudomonas aeruginosa*. These results were agreed with Rosas *et al* who found the isolate of this bacteria were resistant to this antibiotic (Rosas *et al*., 2015). In the current study showed the (Penicillin-G) antibiotic has low activity against *Pseudomonas aeruginosa.* These results were agreed with Javiya *et al* who found this bacteria resistant to many antibiotics such as penicillin-G (Javiya *et al*., 2008). *Pseudomonas aeruginosa* is a major causative agent for nosocomial infections in immunocompromised and burn patients in hospitalized (Biswal *et al*., 2014). This bacteria is have ability to resistant several types of antibiotics because production of antibiotic inactivating enzymes and low permeability of its outer membrane, this expression of various efflux pumps and the (Hillier *et al*., 2006).

In the present study explained the (Amikacin) antibiotic has high activity against *Escherichia coli* (Table:5 and Figure:5.). These results were agreed with Niranjan and Malini who found the isolates of this bacteria were sensitive to this antibiotic (Niranjan and Malini., 2014). But in study of Ruiz *et al* who found some isolates of this bacteria were resistant to this antibiotic (Ruiz *et al*., 2005). The current study explained the (Bacitracin) antibiotic has low activity against *Escherichia coli*. These results were agreed with Wang *et al* who found the isolate of this bacteria were resistant to this antibiotic (Wang *et al*., 2013). In the this study showed the (Rifaximin) antibiotic has low activity against *Escherichia coli.* These results were agreed with Kothary *et al* who found this bacteria resistant to this antibiotic (Kothary *et al*., 2013). *Escherichia coli* is the most common pathogens causing diarrhea with mild to severe watery diarrhea in humans of all ages (Qadri *et al*., 2005). The isolates of this bacteria have ability to resistant of multiple antibiotic types and this has been reported and involving four or more unrelated families of antibiotics, (Maynard *et al*., 2003). This resistance determinants by or mobile genetic elements including transposons, plasmids and generics transformation (Carattoli., 2001).

In the present study showed the (Aztreonam) antibiotic has high activity against *Enterobacter cloacae* (Table:7 and Figure:7.). These results were agreed with Igari who found this antibiotic has activity against this bacteria (Igari., 1994). But dis agreed with Giamarellou *et al* who found the isolate of *Enterobacter cloacae* were resistant to Aztreonam (Giamarellou *et al*., 1989). In the current study showed the (Bacitracin) antibiotic has low activity against *Enterobacter cloacae*. These results were agreed with Sharma *et al* who found the isolates of this bacteria were resistant to this antibiotic (Sharma *et al*., 2015). *Enterobacter cloaca*e have been reported as important opportunistic and multidrug resistant pathogenic bacteria for humans during the last three decades in wards of hospital (Davin-Regli and Pages., 2015). *E.cloacae* causes a wide spectrum of infections involving the urinary tract and several part of human body (Karam and Heffer., 2000). Most isolates of the this bacteria are complex are intrinsically resistant to many types of antibiotics (Stock *et al*., 2001).

In this study explained the (Novobiocin) antibiotic has high activity against *Proteus mirabilis* (Table:9 and Figure:9.). These results were agreed with Safary *et al* who found this antibiotic has high activity against this bacteria (Safary *et al*., 2009). But in study of Al-Mutairi *et al* who found this antibiotic has moderate activity against this bacteria (Al-Mutairi *et al*., 2016). The present study showed the (Penicillin-G) antibiotic has low activity against *Proteus mirabilis*. These results were agreed with Stock who found the isolate of this bacteria were resistant to this antibiotic (Stock., 2003). The Proteus mirabilis are widespread in the environment and most common infections in the human including urinary tract infections (Armbruster and Mobley., 2012). Now the multidrug resistance becoming more common in this bacteria (Mokracka *et al*., 2012).

Figures (1), (2), (4), (6) and (8) explained the antibiotics in this study have different modes of action. These variations deepened on the bacterial types, antibiotic types, mechanisms of bacterial resistant and mode of antibiotics action. However, Antibiotics can be classified based on the system they affect or the cellular component, in addition to whether they induce cell death (bactericidal drugs) or inhibit cell growth (bacteriostatic drugs) (Kohanski *et al*., 2010). In general, most of antibiotics acts by one of the following, inhibit nucleic acids such as inhibit DNA and/or RNA synthesis, replication and joining, inhibit cell wall synthesis and/or breakdown, and/or protein synthesis (Walsh., 2003).

Figure (10) showed the Aztreonam has more activity in comparisons with others. Aztreonam is a bactericidal antibiotic which interferes with the synthesis of the bacterial cell wall (Georgopapadakou *et al*., 1982). This antibiotic has excellent activity against major gram negative pathogens with bactericidal activity (Neu., 1988). Aztrenoam has been used successfully in the treatment of both upper and lower urinary tract infections(Khanb and Shah., 2001). It is has been effective in the treatment of gram negative bacteremia (Scully and Henry., 1985). Bone and Joint Infections caused by susceptible strains of *E. coli*, *Proteus* and *Pseudomonas*

have been treated with Aztreonam (Simons and Lee., 1985). It is useful in treatment of multi drug resistant *Shigella* and *Salmonella* as well as in therapy of gastro intestinal gram negative organisms (Wasfy *et al*., 2000). Aztreonam has been shown to penetrate inflamed meninges, and reach therapeutic levels in the central nervous system (Kapoor and Gathwala., 2004).

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