RESULTS

1 – Isolation of bacterial isolate

In this experiment 50 bacterial isolates were collected from hospitals of Zagazig University. Samples were collected from operating rooms and nursery incubators. Also samples were taken from operating tablets, floor, instruments, beds, windows, doors, lamps and nursery incubator using sterile swaps on nutrients agar as shown in materials and methods . The total bacterial isolates, source and their origin are tabulated in Table (3) .

Table(3) : Total bacterial isolates, source and their origin

Isolate. No	Source	Origin
1	Operating room	Instruments
2	Nursery room	Floor
3	Operating room	Instruments
4	Nursery room	Nursery incubator
5	Operating room	Operating table
6	Operating room	Bed
7	Operating room	Nursery incubator
8	Operating room	Air
9	Nursery room	Instruments
10	Operating room	Instruments
11	Operating room	Instruments
12	Operating room	Air
13	Operating room	Operating table
14	Operating room	Window
15	Operating room	Floor
16	Operating room	Lamp
17	Operating room	Door
18	Operating room	Instruments
19	Operating room	Air
20	Operating room	Instruments
21	Operating room	Bed
22	Operating room	Instruments
23	Operating room	Operating table
24	Operating room	Operating table
25	Operating room	Bed
26	Nursery room	Nursery incubator
27	Nursery room	Instruments
28	Operating room	Operating table
29	Operating room	Instruments
30	Operating room	Instruments
31	Operating room	Bed

32	Operating room	Operating table
33	Operating room	Air
34	Operating room	Instruments
35	Operating room	Instrument
36	Nursery room	Nursery incubator
37	Nursery room	Nursery incubator
38	Operating room	Instruments
39	Operating room	Instruments
40	Operating room	Instruments
41	Operating room	Air
42	Operating room	Floor
43	Operating room	Instruments
44	Operating room	Instruments
45	Nursery room	Floor
46	Operating room	Floor
47	Operating room	Instruments
48	Operating room	Floor
49	Operating room	Window
50	Operating room	Air

2 – Identification of bacterial isolates

Different morphological , physiological and biochemical tests were tabulated in Table (4) . It is clear that the dominant bacterial isolates were *E.coli, Pseudomonas* spp*, Klebsiella, Proteus* spp and *S.aureus* . All the experimental bacterial isolates were non spore forming. All the isolates cells are rods and Grams negative, except *S. aureus* where its cells were clusters of cocci and Grams positive. All bacterial isolates showed positive results with catalase test. *Pseudomonas* spp showed positive results with oxidase test.

All bacterial isolates gave negative result with coagulase test except *S. aureus*. With respect to indole test *E. coil* and *Proteus* spp gave positive results. All bacterial isolates gave positive results with urease test except *E. coil*. with respect to citrate test *Klebsiella* spp, *Pseudomonas* and *Proteus* spp gave positive results while *E. coil* and *S. aureus* gave negative results . All bacterial isolates gave negative results with methyl red test. All bacterial isolates gave positive results with nitrate reduction test while *Pseudomonas* spp dose not. *E. coil, S. aureus* and *Klebsiella* spp could

ferment lactose with acid and gas production while *Pseudomonas* spp and *Proteus* spp were not.

Table (4) : Morphological , physiological and biochemical characters of bacterial isolates

Bacteria / Character	*S.aureus*	*E.coli*	*Klebsiella* spp	*Pseudomonas* Spp	*Proteus* spp
Gram stain	G +ve	G – ve	G – ve	G – ve	G – ve
Shape	Cocci	Rod	Rod	Rod	Rod
Arrangement	Cluster	Monoid or diploid	Monoid or diploid	Straight	Straight
Motility	Non motile	Motile	Non motile	Motile	Motile
Spore forming	Non spore	Non spore	Non spore	Non spore	Non spore
Catalase	+	+	+	+	+
Coagulase	+	-	-	-	-
Oxidase	-	-	-	+	-
Indole	-	+	-	-	+
MR	-	-	-	-	-
VP	-	-	+	+	+
Citrate	-	-	+	+	+
Urease	+	-	+	+	+
Nitrate reduction	+	+	+	-	+

MR = methyl Red Test A = Acid production

V.P = Voges – Proskauer reaction - = Negative , + = Positive

AG = Acid and gas production

Photo (1)

Citrate utilization test (+ve)

Photo (2)

Voges-Proskauer test (+ve)

Photo (3)
Urease test (+ve).

Photo (4)
Carbohydrate fermentation test (+ve)

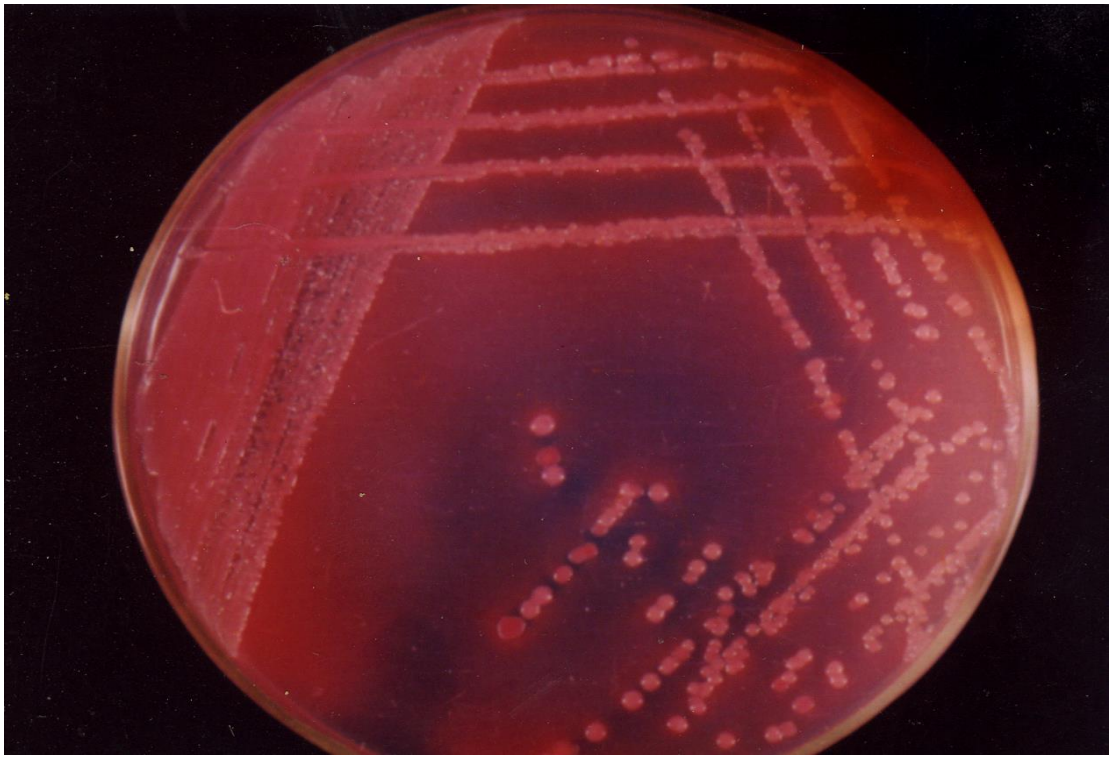


Photo (5) Morphological colony of *Escherichia coli* colonies on MacConkey agar plate

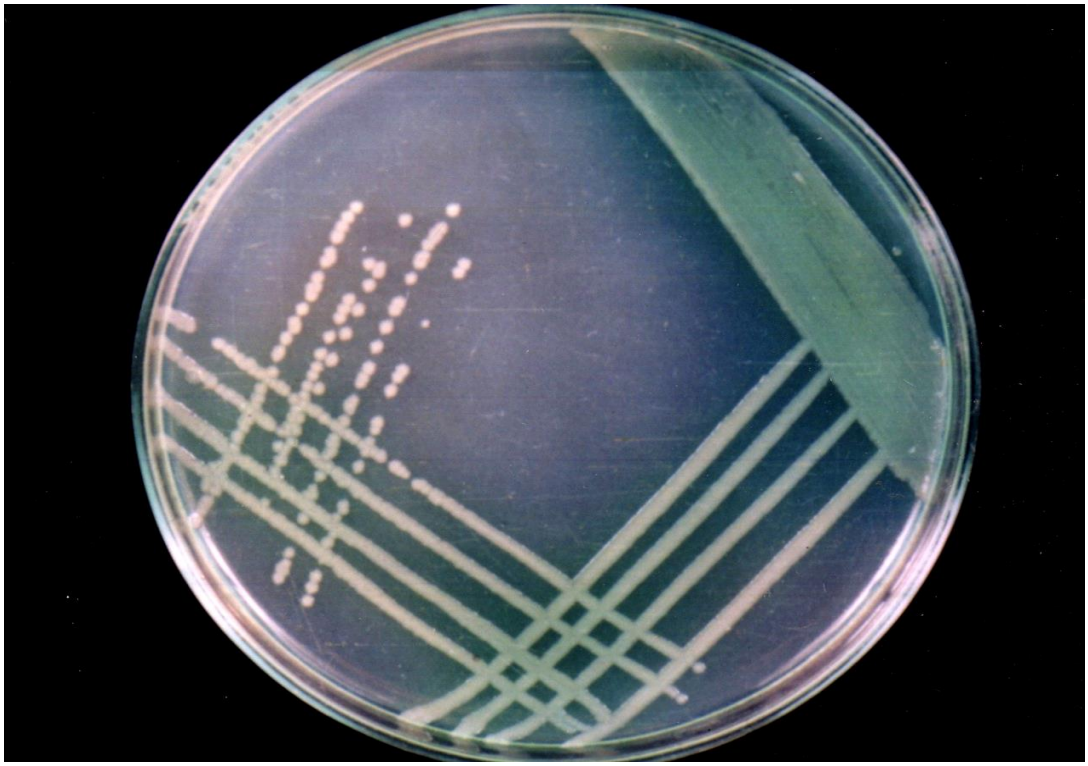


Photo (6) Morphological colony of *Pseudomonas* spp colonies on nutrient agar plate showing greenish-blue pigmentation

3 - Effect antibiotics on bacterial isolates growth

In this experiment, the effect of antibiotics was tested on all the bacterial isolates as illustrated in Tables (5, 6). Impinem showed high effect for both Gram negative and Grams positive bacteria except *E.coli* no (47) which showed intermediate result, choloramephnicol showed 38 resistant and 2 sensitive in case of Gram negative bacteria while gave resistant and 4 sensitive in case of Gram positive. Gentamicin antibiotics show 15 sensitive, 12 intermediates and 13 resistant in Gram negative isolates .

Clindamycin antibiotics had no effect of Gram negative bacteria, ciprofloxacin show high sensitivity for Gram negative bacteria, it was tested on 40 samples of Gram negative bacteria, it showed 35 sensitive and 4 intermediate and 1 resistant. Piperacillin had low sensitive for Gram negative bacteria, it showed 35 resistant, 5 intermediate and 0 sensitive .

Erythomycin had tested for 10 samples Grams positive bacteria show 4 sensitive and 6 resistant. Ofloxacin showed high sensitivity for all Gram positive samples while ampicillin and sulphamethoxazole have not any effect for Gram positive bacteria.

Table (5) : Antibiotics and sensitivity of Gram negative bacteria

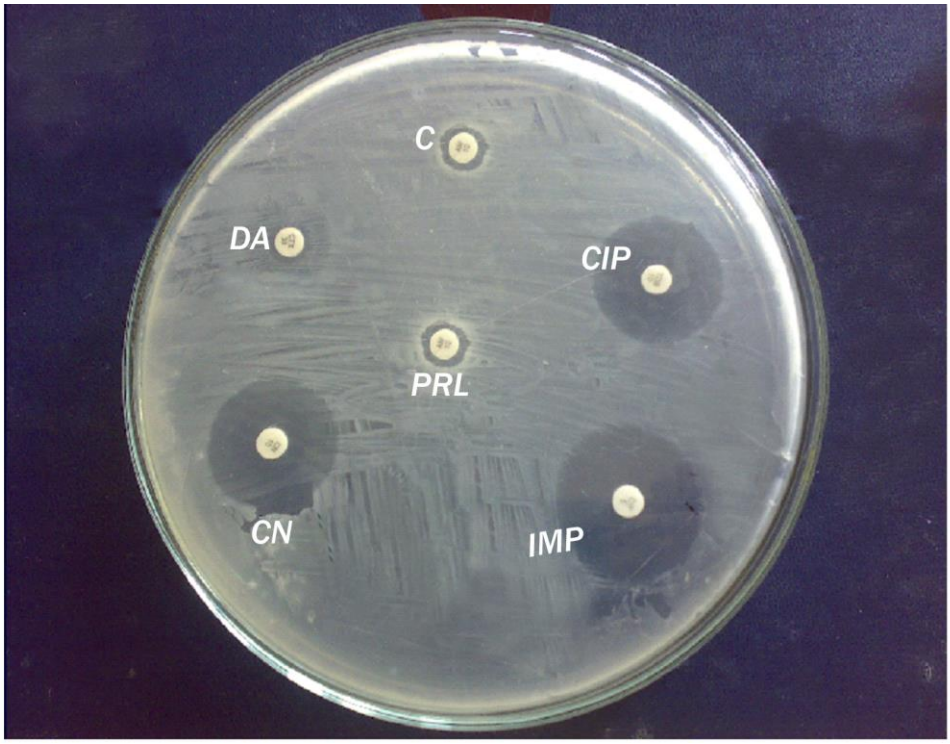
Bacterial Isolates	Diameter of inhibition zones at different antibiotics for G - ve bacteria (mm)								
	IPM	C	CN	DA	CIP	PRL	**S**	**I**	**R**
1 – Klebsiella spp	30(S)	(R)	22(S)	(R)	40(S)	(R)	3	0	3
2 – Klebsiella spp	30(S)	(R)	21(S)	(R)	41(S)	(R)	3	0	3
3 – Klebsiella spp	32(S)	(R)	21(S)	(R)	42(S)	(R)	3	0	3
4 – Klebsiella spp	30(S)	(R)	23(S)	(R)	36(S)	(R)	3	0	3
5 – Klebsiella spp	22(S)	(R)	23(S)	(R)	22(S)	(R)	3	0	3
6 – Klebsiella spp	30(S)	(R)	22(S)	(R)	40(S)	(R)	3	0	3
7 – Klebsiella spp	30(S)	(R)	22(S)	(R)	30(S)	(R)	3	0	3
8 – E.coli	30(S)	(R)	21(S)	(R)	30(S)	(R)	3	0	3
9 – E.coli	30(S)	(R)	14(I)	(R)	31(S)	(R)	2	1	3
10 – E.coli	30(S)	(R)	13(I)	(R)	30(S)	(R)	2	1	3
11– E.coli	34(S)	(R)	10(R)	(R)	30(S)	(R)	2	0	4
12 – E.coli	30(S)	(R)	10(R)	(R)	38(S)	(R)	2	0	4
13– E.coli	30(S)	(R)	10(R)	(R)	30(S)	(R)	2	0	4
14 – E.coli	40(S)	(R)	14(I)	(R)	30(S)	(R)	2	1	3
15 – E.coli	38(S)	(R)	14(I)	(R)	30(S)	(R)	2	1	3
16 – E.coli	30(S)	(R)	22(S)	(R)	30(S)	(R)	3	0	3
17 – E.coli	30(S)	(R)	14(I)	(R)	30(S)	(R)	2	1	3
18 – E.coli	40(S)	(R)	14(I)	(R)	40(S)	(R)	2	1	3
19 – E.coli	30(S)	(R)	23(S)	(R)	38(S)	(R)	3	0	3
20 – Pseudomonas spp	39(S)	(R)	24(S)	(R)	38(S)	(R)	3	0	3
21 – Pseudomonas spp	30(S)	(R)	10(R)	(R)	30(S)	(R)	2	0	4
22– Pseudomonas spp	30(S)	(R)	13(I)	(R)	30(S)	(R)	2	1	3
23 – Pseudomonas spp	36(S)	(R)	10(R)	(R)	25(S)	(R)	2	0	4
24 – Pseudomonas spp	26(S)	(R)	14(I)	(R)	26(S)	(R)	2	1	3
25 – Pseudomonas spp	26(S)	(R)	14(I)	(R)	27(S)	(R)	2	1	3
31 – Proteus spp	28(S)	(R)	(R)	(R)	29 (S)	22(I)	2	1	3
32 – Proteus spp	30(S)	(R)	(R)	(R)	28(S)	20(I)	2	1	3
33 – Proteus spp	27(S)	(R)	(R)	(R)	24(S)	20(I)	2	1	3
34 – Proteus spp	32(S)	(R)	(R)	(R)	20(I)	(R)	1	1	4
35 – Proteus spp	24(S)	(R)	(R)	(R)	20(I)	20(I)	1	2	3
37 – Klebsiella spp	34(S)	(R)	14(I)	(R)	40(S)	(R)	2	1	3
39 – Pseudomonas spp	38(S)	(R)	20(S)	(R)	36(S)	(R)	3	0	3
40 – Klebsiella spp	30(S)	(R)	20(S)	(R)	20(I)	(R)	2	1	3
42 – Pseudomonas spp	30(S)	(R)	10(R)	(R)	30(S)	(R)	2	0	4
43 – Pseudomonas spp	28(S)	(R)	10(R)	(R)	(R)	(R)	1	0	5
45 – Klebsiella spp	30(S)	(R)	14(I)	(R)	25(S)	20(I)	2	2	2
47 – E.coli	15(I)	28(S	21(S)	(R)	20(I)	(R)	2	2	2
48 – Klebsiella spp	31(S)	(R)	21(S)	(R)	26(S)	(R)	3	0	3

49 – E.coli	30(S)	(R)	11(R)	(R)	28(S)	(R)	2	0	4
50 – Klebsiella spp	30(S)	30(S	20(I)	(R)	28(S)	(R)	3	1	2
S	39	2	15	0	35	0	91		
I	1	0	12	0	4	5		22	
R	0	38	13	40	1	35			127
							91	22	127

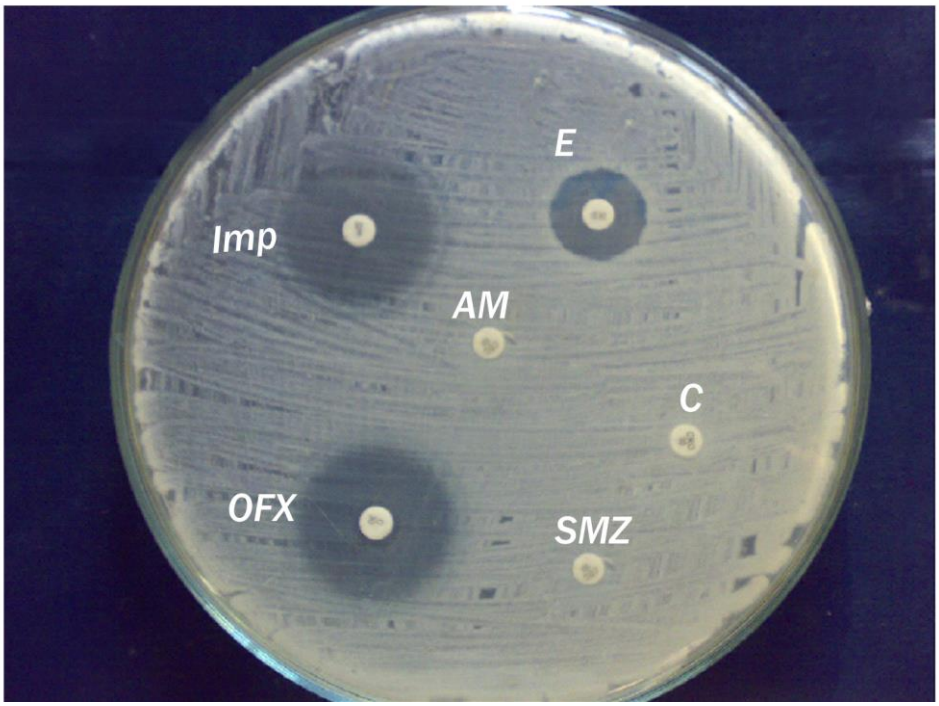
Table (6) : Antibiotics and sensitivity of Gram Positive bacteria

Bacteria isolated	Diameter of inhibition zones at different antibiotics for G +ve bacteria (mm)								
	IPM	E	CN	AM	OFX	SMZ	**S**	**I**	**R**
26 – S.aureus	40(S)	(R)	30(S)	(R)	29(S)	(R)	3	0	3
27 – S.aureus	40(S)	(R)	32(S)	(R)	30 (S)	(R)	3	0	3
28 – S.aureus	39(S)	(R)	30(S)	(R)	38(S)	(R)	3	0	3
29 – S.aureus	36(S)	(R)	31(S)	(R)	30(S)	(R)	3	0	3
30 – S.aureus	40(S)	(R)	25(S)	(R)	30(S)	(R)	3	0	3
36 – S.aureus	40(S)	(R)	25(S)	(R)	26(S)	(R)	3	0	3
38 – S.aureus	30(S)	18(S)	20(I)	(R)	25(S)	(R)	3	1	2
41 – S.aureus	40(S)	30(S)	25(S)	(R)	26(S)	(R)	4	0	2
44 – S. aureus	40(S)	30(S)	24(S)	(R)	26(S)	(R)	4	0	2
46 – S.aureus	36(S)	30(S)	30(S)	(R)	25(S)	(R)	4	0	2
S	10	4	9	0	10	0	33		
I	0	0	1	0	0	0		1	
R	0	6	0	10	0	10			26
							33	1	26

IPM = Imipenem
C = Chloramephnicol
CN = Gentamicin
DA = Clindamycin
CIP = Ciprofloxacin
PRL = Piperacillin
E = Erythromycin
AM = Ampicillin
OFX = Ofloxacin
SMZ = Sulphamethoxazole
S = Sensitive
I = Intermediate
R = Resistance



E.coli (16)



Staph aureus (30)

Photo (7) : Antibacterial activity of antibiotics

3.1Suceptibility of Gram negative and Gram positive bacterial isolates to antibiotic

Impinem and ciprofloxacin antibiotics show high sensitive to different Gram positive bacterial isolates with percentage 97.5, 87.5 respectively while gentamicin show moderate sensitive to same isolates with percentage 32.5 % . Piperacillin , clindamycin and chloramephnicol have few or no effect on Gram negative isolates with percentage 2.5 . 0.5 % respectively .

Impinem and ofloxacin antibiotics show high sensitive to different Gram positive bacterial isolates with percentage 100 % for both. chloramephnicol show moderate sensitive to same isolates with percentage 60 % . Ampicillin, sulphamethoxazole and erythromycin have weak effect on Gram positive isolates .

Table (7) : Susceptibility of Gram negative bacterial isolates to Antibiotics

Antibiotics / Activity	Impinem	Chloramephnicol	Gentamicin	Clindamycin	Ciprofloxacin	Piperacillin
Resistant %	0	95	37.5	100	2.5	87.5
Moderately %	2.5	0	30	0	10	10
Sensitive %	97.5	5	32.5	0	87.5	2.5

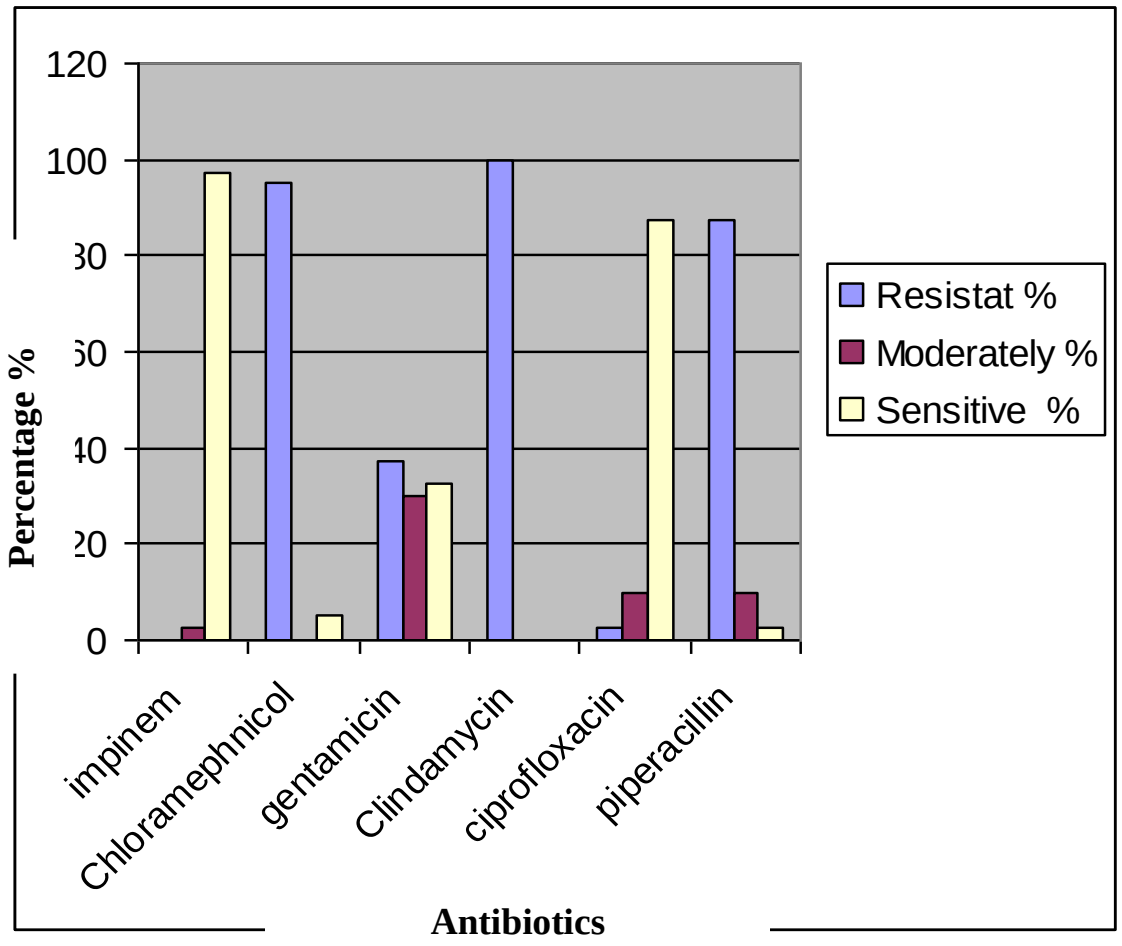


Figure (1) : Susceptibility of Gram negative bacterial isolates to antibiotic

Table (8) : Susceptibility of Gram Positive bacterial isolates to antibiotics

Antibiotics / Activity	Impinem	Chloramephnicol	Ampicillin	Sulphamethoxazole	Ofloxacin	Erythromycin
Resistant %	0	40	10	100	0	90
Moderately %	0	0	0	0	0	10
Sensitive %	100	60	0	0	100	0

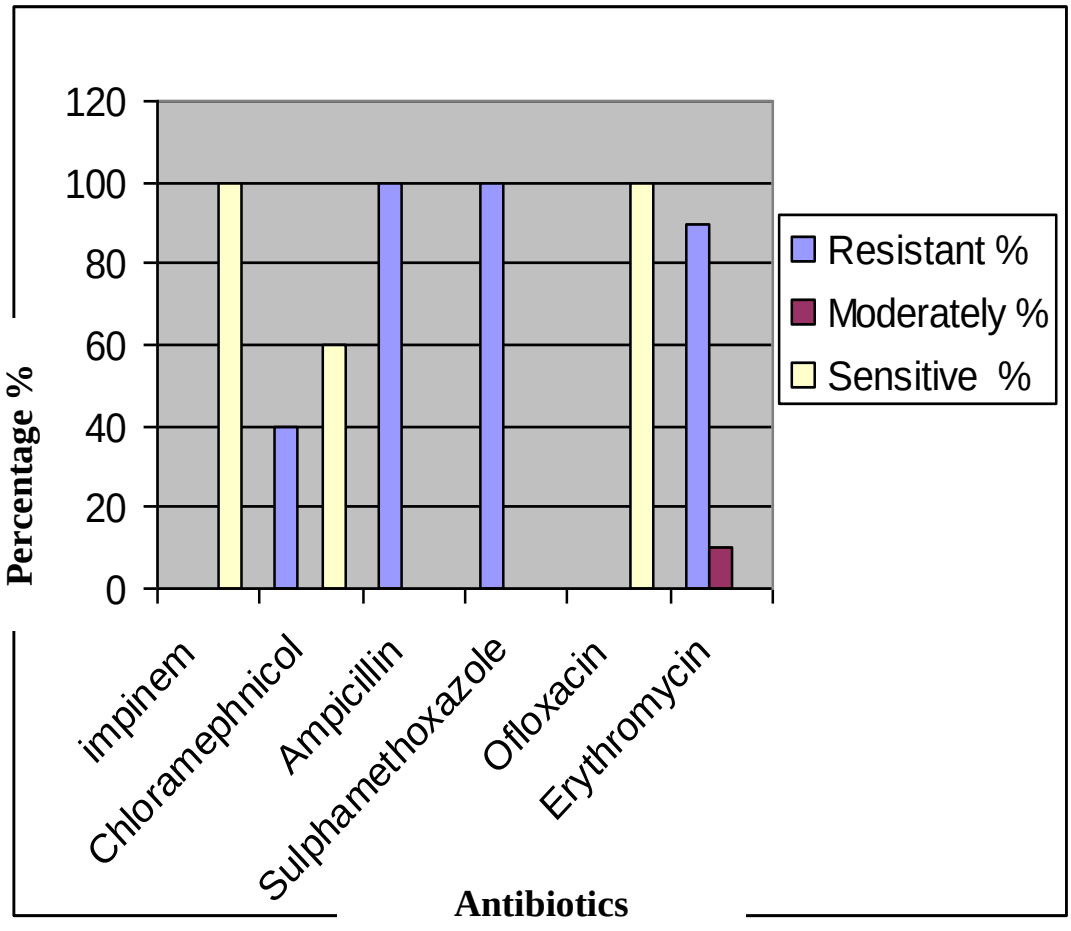


Figure (2): Susceptibility of Gram Positive bacterial isolates to antibiotics

3.2. Determination of MIC and MBC for different bacterial isolates

In this experiment, the minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) of impinem and ciprofloxacin were determined for *E.coli* , *Pseudomonas* spp and *Proteus* spp. The obtained results in Tables (9,10), clearly illustrate that the impinem was active than ciprofloxacin against different *E.coli* isolates hence it gave lowest values of MIC and MBC than ciprofloxacin, MIC gave values (20-30 ug/ml) for ciprofloxacin but gave values (10-20 ug/ml) for impinem against *E.coli* isolates.

It is clear also that impinem was active against *Pseudomonas* spp than ciprofloxacin hence it gave lowest values than ciprofloxacin MIC gave values. The results showed that MIC values for *Pseudomonas* spp no 21,23,42 and 43 were 20,30,30 and 30 ug/ml respectively in case of ciprofloxacin and the same isolates gave MBC values 20,30,20 and 20 ug/ml in case of impinem used.

The same action of both impinem and ciprofloxacin occurs against *Proteus* spp isolates MIC values for *Proteus* spp was (30 ug/ml) for ciprofloxacin and (20 ug/ml) for impinem . Generally from this results it is obvious that MIC values for impinem less than ciprofloxacin so impinem become more effective than ciprofloxacin for *E.coli , Proteus* spp and *Pseudomonas* spp .

Table(9) MIC and MBC (ug/ml) of ciprofloxacin against selected clinical bacterial isolates

Isolate .no	MIC (ug/ml)	MBC (ug/ml)
E.coli (11)	20	30
E.coli (12)	20	20
E.coli (13)	20	30
Pseudomonas spp (21)	20	30
Pseudomonas spp (23)	30	40
Proteus spp (34)	30	30
Proteus spp (35)	30	40
Pseudomonas spp (42)	30	40
Pseudomonas spp (43)	30	40
E.coli (49)	30	40

Table(10) MIC and MBC (ug/ml) of impinem against selected clinical bacterial isolates

Isolate .no	MIC (ug/ml)	MBC (ug/ml)
E.coli (11)	10	20
E.coli (12)	20	20
E.coli (13)	10	20
Pseudomonas spp (21)	20	30
Pseudomonas spp (23)	30	30
Proteus spp (34)	20	30
Proteus spp (35)	20	30
Pseudomonas spp (42)	20	30
Pseudomonas spp (43)	20	30
E.coli (49)	10	20

4 - Effect of different essential oils on the bacteria isolates : -

In this experiment, the effect of ten (thyme, caraway, clove, garlic ,lemon, peppermint, jasmine, anise, rosemary and chamomile) **,** essential oils were studied on both Gram negative and Gram positive bacteria , the obtained results are tabulated in tables (11,12). It is obvious from the results that thyme and rosemary inhibit the growth of Gram negative bacteria, caraway oil have no effect on *Pseudomonas* spp and have weak effect on another Grams negative bacteria where chamomile have not any effect on Gram negative of bacterial isolates. Thyme, cloves, pepper, rosemary and chamomile inhibit growth of *S.aureus* while caraway, lemon , jasmine and anise have low effect on *S.aureus* while garlic had no effect on *S.aureus.*

Table (11) : Effect of different oils on the growth of Gram negative bacterial isolates

Bacterial Isolates	Diameter of inhibition zones at different essential oils for G - ve bacteria (mm)									
	Thyme	Caraway	Cloves	Garlic	Lemon	Peppermint	Jasmine	Anise	Rosemary	Chamomile
1 – Klebsiella spp	20	0	0	0	0	0	4	0	18	0
2 – Klebsiella spp	18	10	0	0	0	0	9	0	20	0
3 – Klebsiella spp	0	7	0	0	0	0	4	0	16	8
4 – Klebsiella spp	22	14	0	0	8	0	0	0	17	0
5 – Klebsiella spp	21	13	0	0	9	0	8	8	17	7
6 – Klebsiella spp	20	8	0	0	9	0	9	9	18	0
7 – Klebsiella spp	0	8	0	0	0	0	0	0	15	0
8 – E.coli	25	9	10	0	0	13	4	0	14	0
9 – E.coli	30	0	12	0	0	11	5	0	17	0
10 – E.coli	26	0	15	0	0	12	9	0	14	0
11– E.coli	8	16	12	0	11	12	5	0	16	9
12 – E.coli	24	5	12	8	12	15	1	9	16	0
13– E.coli	29	4	11	6	12	17	0	7	16	0
14 – E.coli	25	5	15	0	0	14	0	6	15	0
15 – E.coli	7	6	15	0	0	14	7	0	19	0
16 – E.coli	22	19	11	0	0	15	4	0	12	0
17 – E.coli	5	0	13	6	0	13	9	0	16	0
18 – E.coli	26	0	16	5	13	12	4	0	16	0
19 – E.coli	30	8	11	0	11	15	0	0	17	0
20 – Pseudomonas spp	30	0	0	0	0	0	0	0	8	0
21 – Pseudomonas spp	16	0	20	0	0	0	0	0	12	0
22– Pseudomonas spp	21	0	12	0	0	0	0	0	18	0
23 –Pseudomonas spp	0	0	10	0	0	0	0	0	16	0
24 – Pseudomonas spp	0	0	14	0	0	0	0	0	0	0
25 – Pseudomonas spp	18	0	14	0	0	0	0	0	13	0
31 – Proteus spp	0	5	9	0	0	10	4	0	12	0
32 – Proteus spp	19	3	10	0	0	8	9	0	10	0
33 – Proteus spp	20	4	8	0	0	11	4	0	8	0
34 – Proteus spp	21	5	12	0	0	12	0	0	15	0
35 – Proteus spp	0	5	11	0	0	10	8	0	13	0
37 – Klebsiella spp	18	0	14	0	0	0	9	0	0	0
39 – Pseudomonas spp	23	0	18	0	0	0	0	0	12	0
40 – Klebsiella spp	25	0	0	0	0	0	4	10	0	0
42 – Pseudomonas spp	21	0	0	0	0	0	5	0	10	0
43 – Pseudomonas spp	0	0	16	0	0	0	9	0	10	0
45 – Klebsiella spp	22	0	12	0	0	0	5	10	11	0
47 – E.coli	23	0	13	0	10	11	1	0	0	0
48 – Klebsiella spp	21	0	11	0	0	0	0	0	12	0
49 – E.coli	24	0	11	7	7	12	12	0	14	0

50 – Klebsiella spp	25	0	14	0	7	0	17	0	14	0

Table (12) : Effect of different oils on the growth of Gram positive bacterial isolates

Bacterial Isolates	Diameter of inhibition zones at different essential oils mm for G +ve bacteria									
	Thyme	Caraway	Cloves	Garlic	Lemon	Peppermint	Jasmine	Anise	Rosemary	Chamomile
26 – S.aureus	22	0	25	0	0	20	11	10	29	18
27 - S.aureus	21	0	22	0	9	18	8	9	30	19
28 - S.aureus	21	12	20	0	9	20	8	12	31	18
29 - S.aureus	8	17	24	0	0	15	0	11	29	16
30 - S.aureus	0	10	26	0	0	24	0	0	28	21
36 – S.aureus	25	0	25	0	5	24	6	0	30	22
38 – S.aureus	25	0	21	0	6	22	0	0	30	23
41 – S.aureus	12	9	21	0	0	17	3	7	27	22
44 - S. aureus	22	10	23	0	0	21	12	9	29	24
46 – S.aureus	24	9	24	0	7	25	8	13	28	21

(A) : Antibacterial activity of essential oils against *E.coli*
1 = Thyme , 2 = Garlic , 3 = Jasmine , 4 = Chamomile , 5 = Caraway , 6 = Clove , 7 = Peppermint , 8 = Rosemary , 9 = Anise , 10 = Lemon

(B) : Antibacterial activity of essential oils against *Pseudomonas* spp
1 = Clove , 2 = Thyme , 3 = Jasmine , 4 = Rosemary , 5 = Carawy , 6 = Chamomile , 7 = Garlic , 8 = Lemon, 9 = Peppermint , 10 = Anise

Photo (8) : Antibacterial activity of essential oils

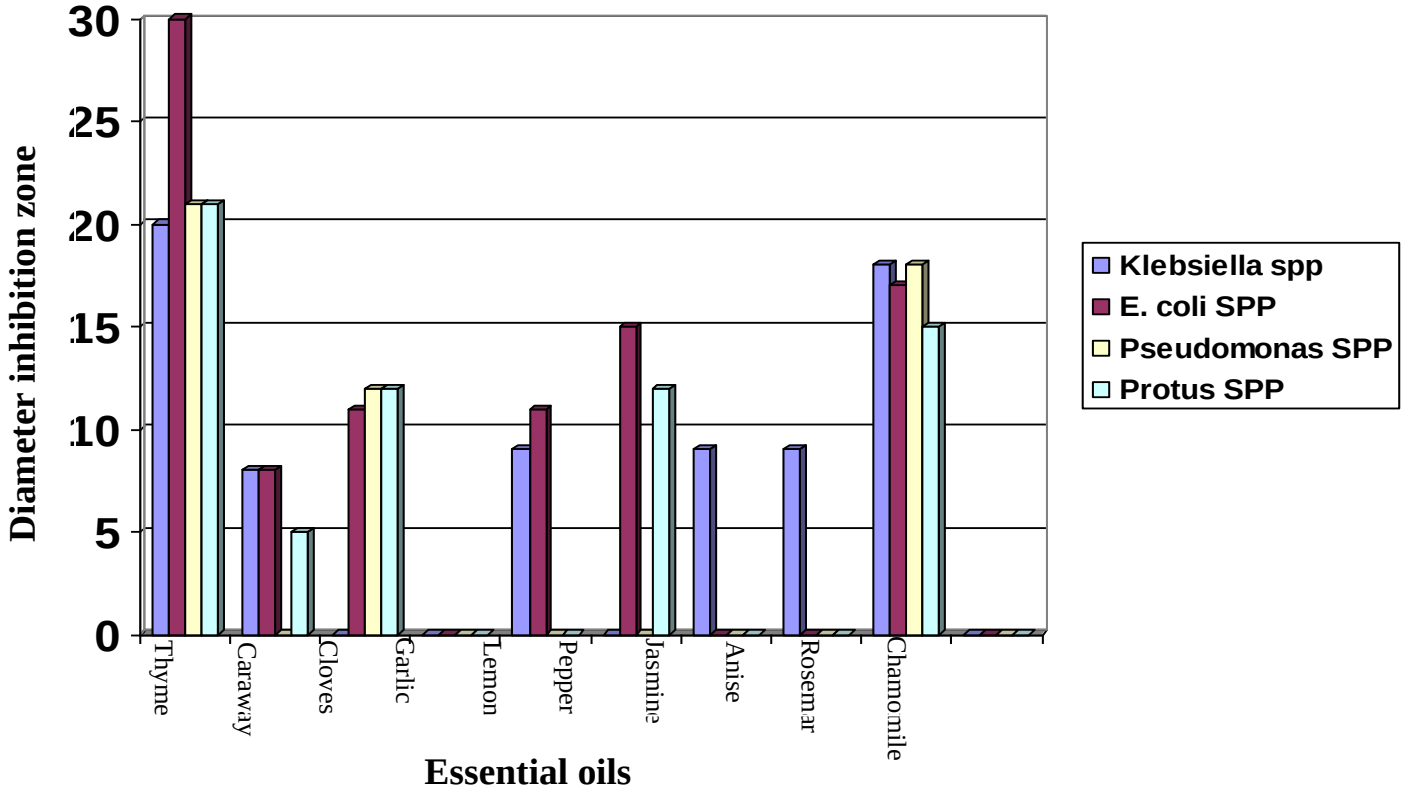


Figure (3) :Antibacterial action of different essential oils on G –ve bacteria

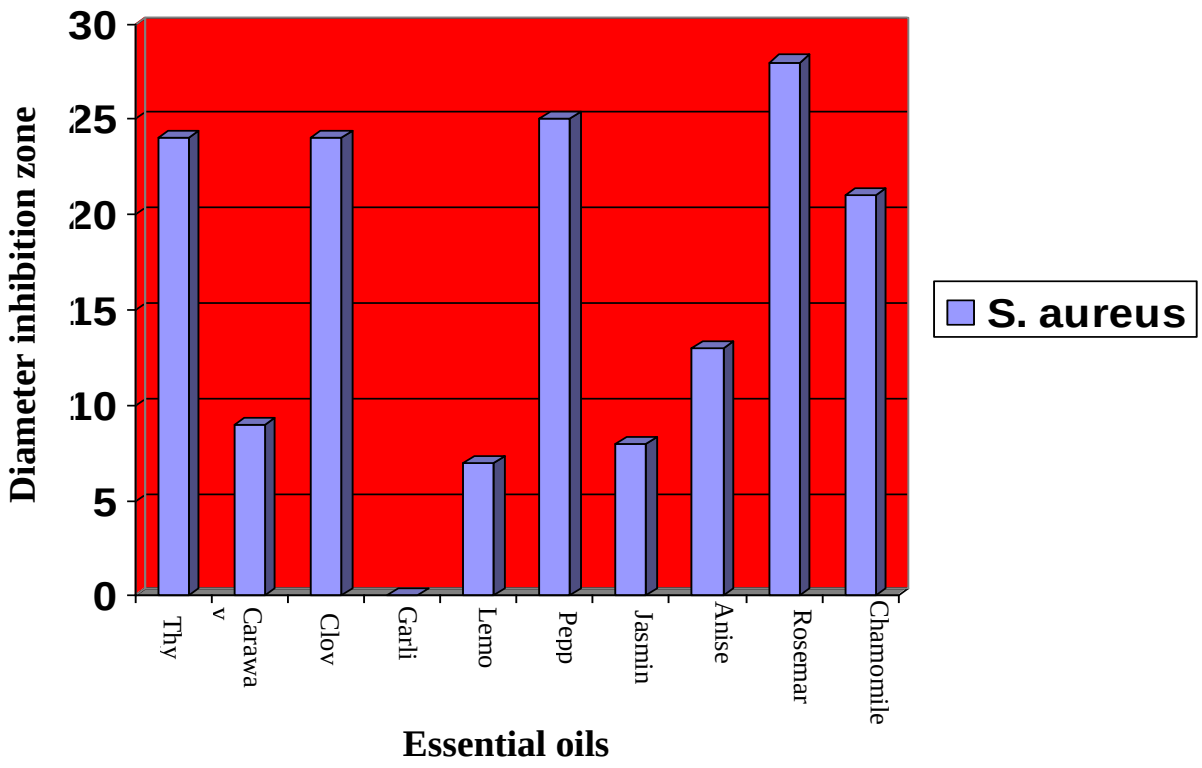


Figure (4) :Antibacterial action of different essential oils on G +ve bacteria

5 - Combination between antibiotics and essential oils

The effect of combination between most effective antibiotics and most active essential oil against Gram positive and Gram negative bacterial isolates were evaluated to study the synergetic or antagonistic effect of combination between antibiotics as impinem and ciprofloxacin and essential oils as thyme and rosemary compared with effective of antibiotics and oils alone . The obtained results in Table (13), photo (9) and figures (5,6), clearly illustrated that the combination between impinem and ciprofloxacin with both thyme and rosemary gave highly synergetic effect against Gram negative bacterial isolates especially *E.coli* no (13) and (49) . Also combination between impinem and ofloxacin with chamomile and clove gave highly effect against Gram positive bacterial isolates especially *S.aureus* no (44) .

Table (13) Combination between antibiotics and essential oils

Bacterial isolates	Diameter of inhibition zones for G -ve bacteria (mm)					
	IMP	IMP + Thyme	IMP + Rosemary	CIP	Cip + Thyme	Cip + Rosemary
E.coli (11)	34	38	35	30	31	34
E.coli (12)	30	32	32	38	38	40
E.coli(13)	30	40	31	30	32	37
Pseudomonas spp (21)	30	35	30	30	35	31
Proteus spp (34)	32	36	32	20	20	32
Pseudomonas spp (42)	30	32	30	30	31	30
Pseudomonas spp (43)	28	28	28	0	0	8
E.coli (49)	30	37	30	28	32	34
Bacterial Isolates	Diameter of inhibition zones for G +ve bacteria (mm)					
	IMP	IMP + Clove	IMP + Chamomile	OFX	OFX + Clove	OFX + Chamomile
S.aureus (38)	38	39	32	25	30	28
S.aureus (41)	40	47	40	26	30	32
S.aureus (44)	40	47	42	26	29	29
S.aureus (46)	36	39	38	25	34	33

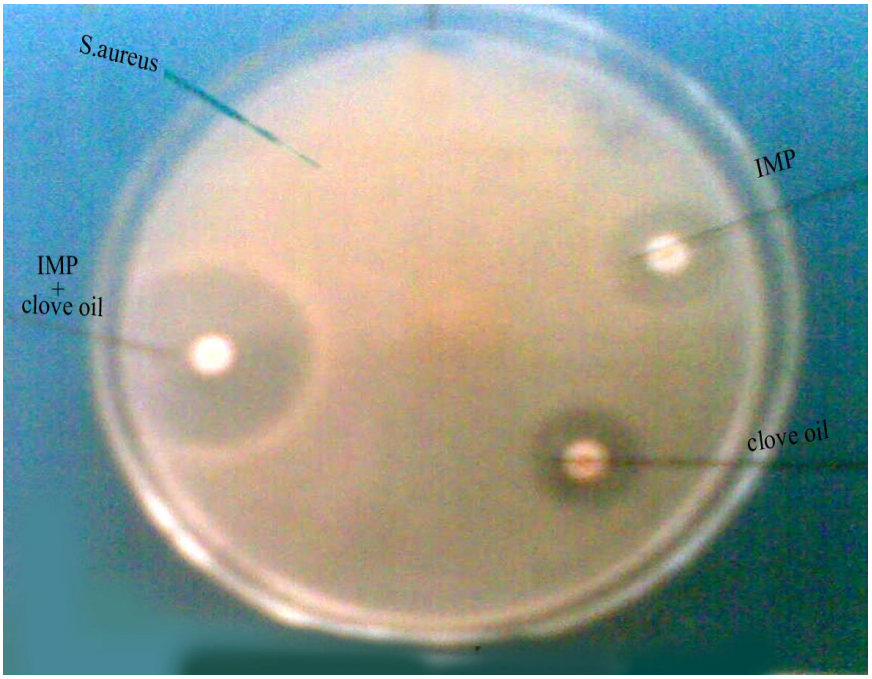


Photo (9) :Infleunce of combination between Impinem and clove oil on *S.aureus* (44)

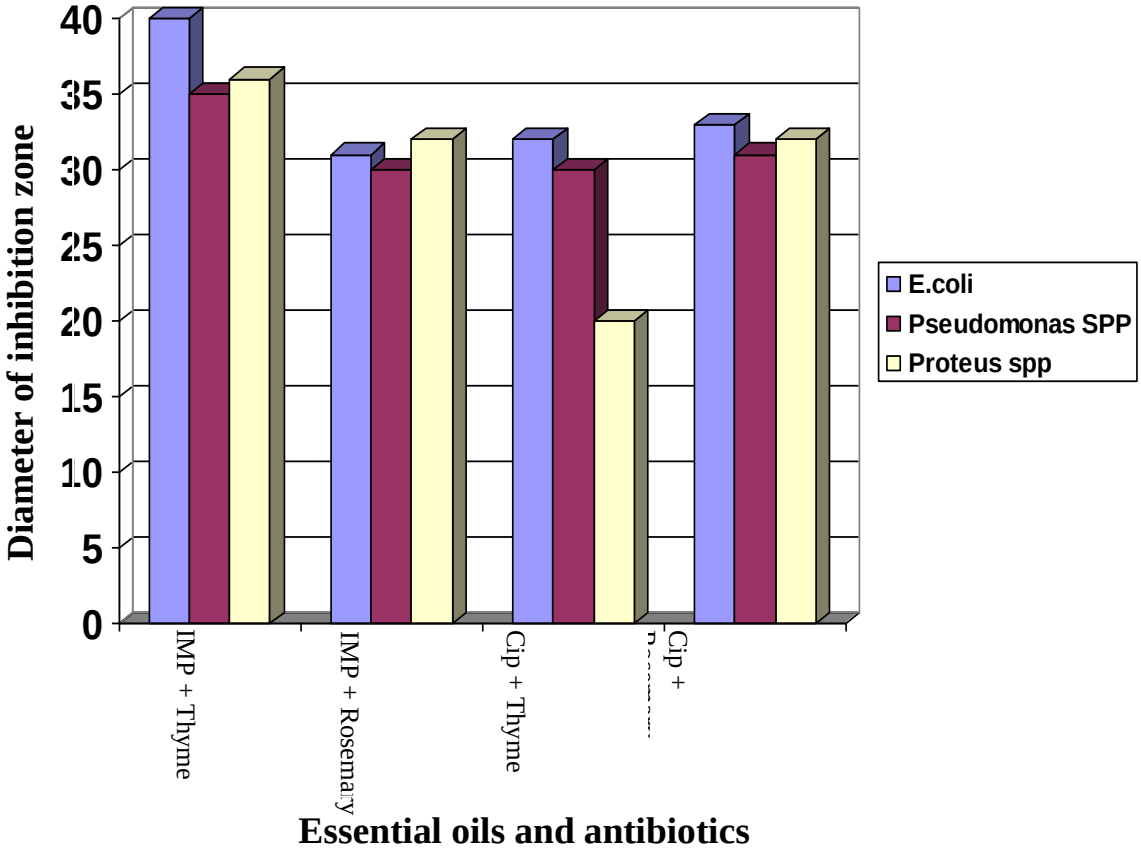


Figure (5) :Antibacterial action of combination essential oils with antibiotics on G -ve bacteria

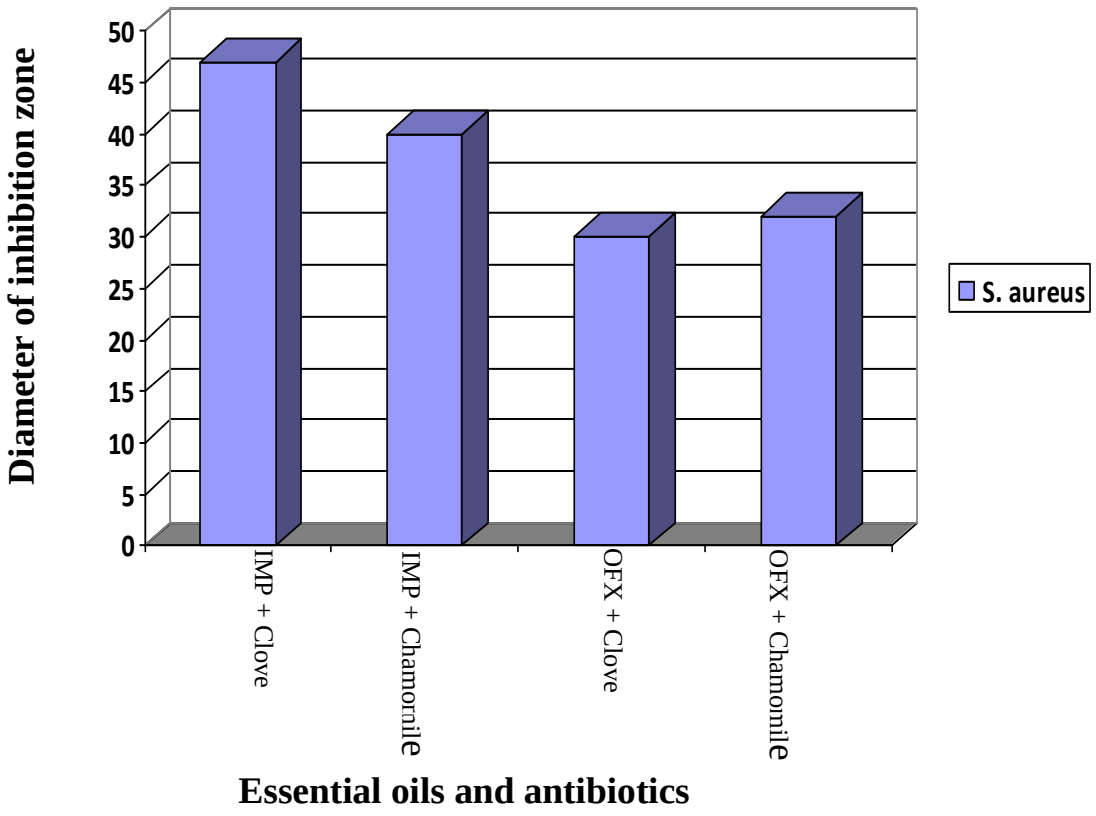
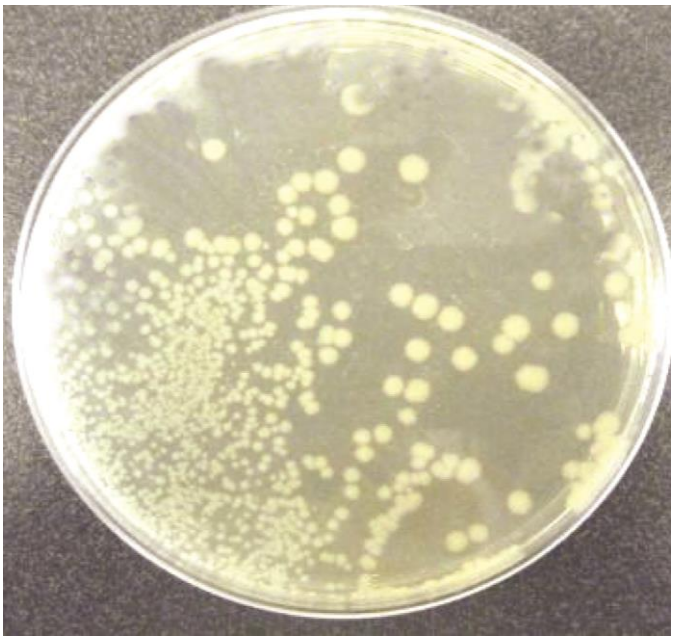


Figure (6) : Antibacterial action of combination essential oils with antibiotics on G +ve bacteria

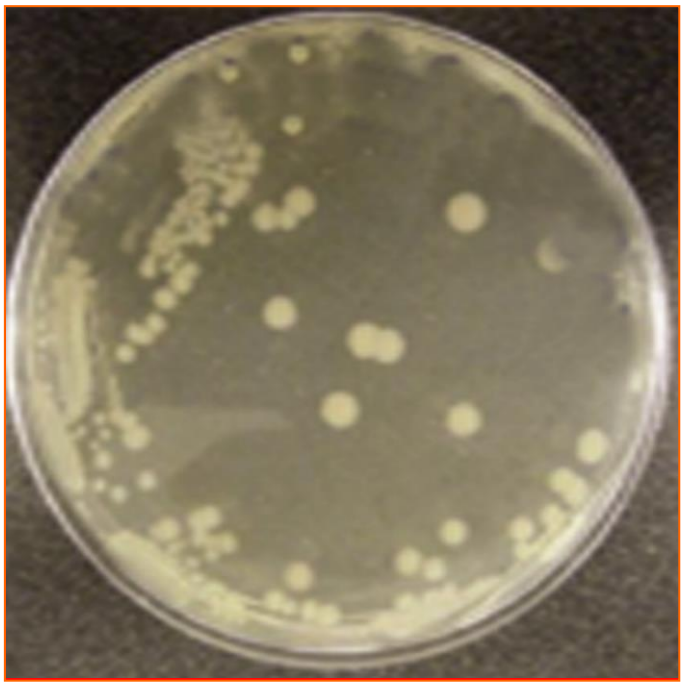
6 – Effect of radiations on the most resistant bacterial strains

6.1. Antibacterial activity of ultraviolet rays

In this experiment, the effect of ultraviolet rays (254 nm) was studied on the survival of bacterial isolates of viable cells after exposure for different periods (0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 ,11 ,12 minutes) at 20 cm distance from UV lamp . The obtained results are demonstrated in Table (14) , photo (10) and figures (7,8) which showed that the elapsing of the exposure time to short wavelength ultraviolet (254 nm) at the distance of 20 cm caused a decrease in the percentage of survival of cells .This decrease with increasing the exposure time and the total viable counts for *E.coli* no (11) and (12) complete inhibited after (11) minutes of exposure to UV . *Proteus* spp complete inhibited after 9 minutes, *Pseudomonaus* spp no (21), (42) after 11 minutes and *E.coli* no (49) after 10 minutes, This means that the majority of cells failed to survive after the first 8 minutes of exposure.



E.coli **after 2 minutes of UV rays**



E.coli **after 7 minutes of UV rays**

Photo (10): Antibacterial activity of ultraviolet rays on *E.coli*

Table (14) : Effect of Ultraviolet rays (254 nm) on some bacterial isolates .

Bacterial isolates	Time (min)	No of cells	log no of cells	Bacterial isolates	Time (min)	No. of cells	log no of cells
E.coli(11)	0	12×10^8	9.1	*E.coli (12)*	0	16×10^8	9.2
	1	10×10^7	8		1	14×10^8	9.1
	2	10×10^6	7		2	12×10^6	7.1
	3	9×10^5	5.9		3	8×10^5	5.9
	4	9×10^3	3.9		4	8×10^3	3.9
	5	3×10^3	3.5		5	3×10^3	3
	6	10×10^2	3		6	8×10^2	2.9
	7	8×10^2	2.9		7	2×10^2	2.3
	8	6×10^2	2.8		8	144	2.1
	9	70	1.8		9	90	1.9
	10	30	1.5		10	60	1.8
	11	0	0		11	0	0
	12	0	0		12	0	0
E.coli (13)	0	25×10^8	9.4	*Pseudomonas* spp (21)	0	34×10^8	9.5
	1	14×10^8	9.1		1	22×10^7	8.3
	2	10×10^7	8		2	10×10^7	8
	3	2×10^7	7.3		3	28×10^6	7.4
	4	8×10^6	6.9		4	25×10^5	6.4
	5	5×10^6	6.7		5	9×10^5	5.9
	6	2×10^4	4.3		6	4×10^4	4.6
	7	9×10^3	3.9		7	7×10^3	3.8
	8	2×10^2	2.3		8	2×10^3	3.3
	9	50	1.7		9	130	2.5
	10	0	0		10	70	1.85
	11	0	0		11	0	0
	12	0	0		12	0	0
Proteus spp (34)	0	26×10^7	8.4	*Pseudomonas* spp (42)	0	30×10^8	9.5
	1	18×10^7	8.2		1	22×10^8	9.3
	2	9×10^6	6.9		2	5×10^8	8.6
	3	5×10^5	5.6		3	21×10^7	8.3
	4	4×10^3	3.6		4	25×10^6	7.4
	5	8×10^2	2.9		5	9×10^5	5.9
	6	2×10^2	2.3		6	2×10^4	4.3
	7	129	2.1		7	7×10^3	3.8
	8	55	1.7		8	4×10^2	2.6
	9	0	0		9	150	2.2
	10	0	0		10	70	1.8
	11	0	0		11	0	0
	12	0	0		12	0	0
Pseudomonas spp (42)	0	33×10^7	8.5	*E.coli* (49)	0	15×10^8	9.2
	1	31×10^6	7.5		1	8×10^7	7.9
	2	24×10^5	6.4		2	13×10^6	7.1
	3	21×10^5	6.3		3	6×10^5	5.8

	4	3×10^{4}	4.5		4	6×10^{4}	4.8
	5	9×10^{3}	3.9		5	3×10^{3}	3.5
	6	2×10^{3}	3.3		6	7×10^{2}	2.8
	7	14×10^{2}	9.2		7	145	2.2
	8	4×10^{2}	2.6		8	60	1.8
	9	144	2.15		9	23	1.4
	10	0	0		10	0	0
	11	0	0		11	0	0
	12	0	0		12	0	0

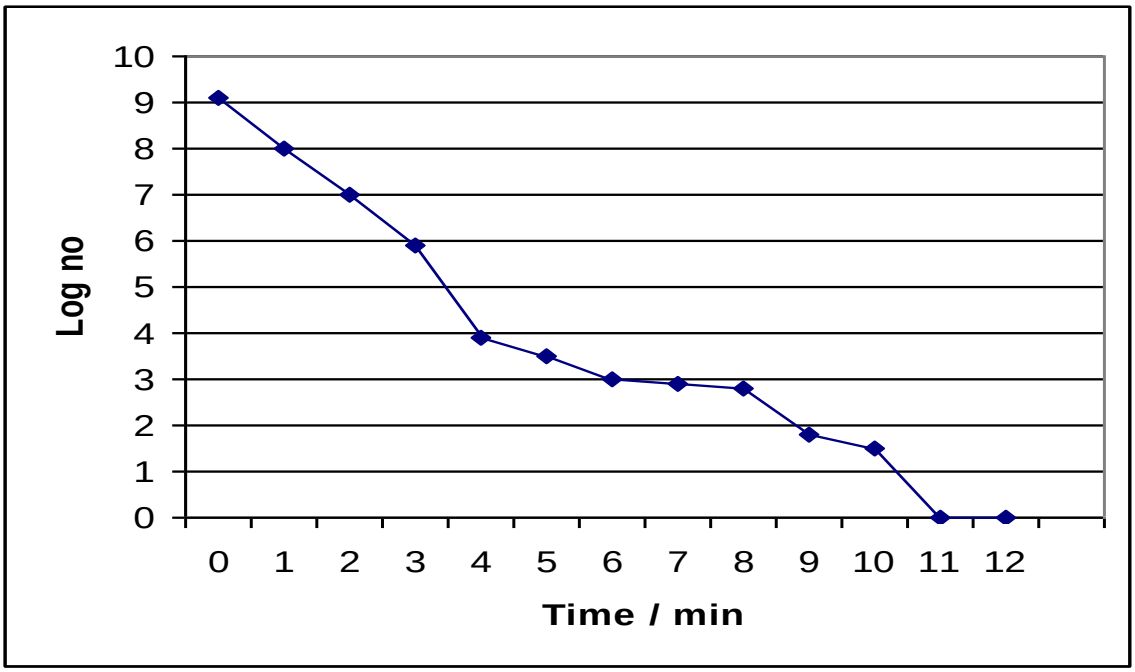


Figure (7): Dose survival curve of *E.coli* (11) viable cells after exposure to UV rays (254 nm) for different periods .

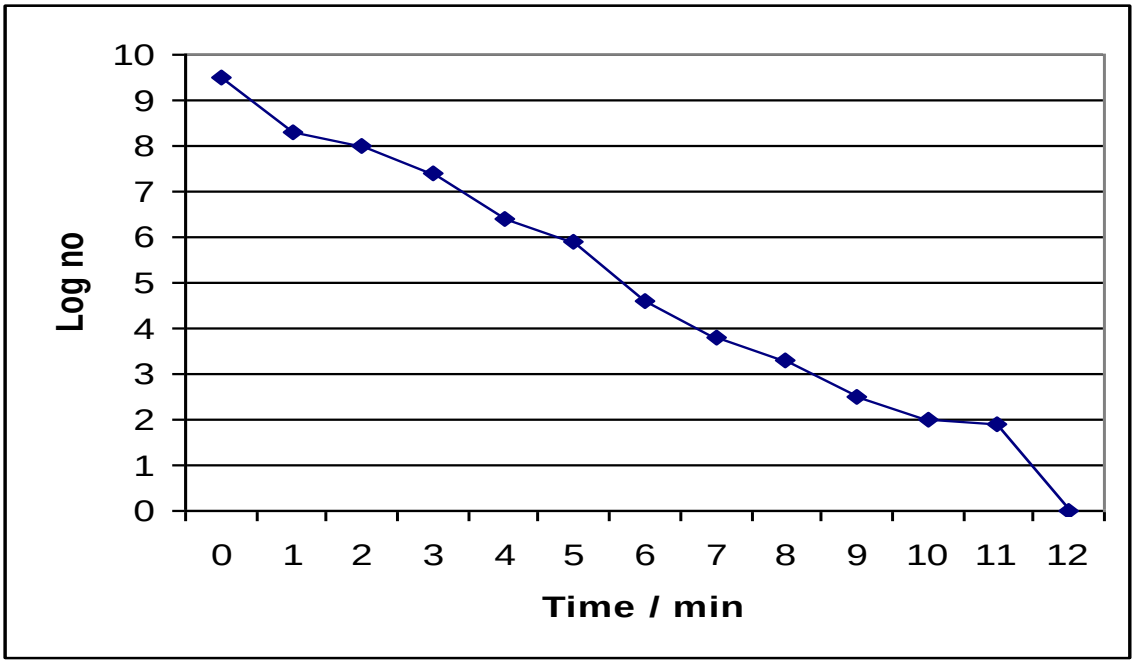


Figure (8): Dose survival curve of *Pseudomonas* spp (21) viable cells after exposure to UV rays (254 nm) for different periods .

6.2. Antibacterial activity of gamma irradiation

The result of survival of the subjected viable cells are shown in table (15) and figures (9) and (10). these results showed that increasing of gamma radiation doses decreased that total number of viable bacterial cells and the total viable counts for *E.coli* no (11), (12) ,(13) and (49) were completely inhibited after 3 KGy , *Proteus* spp complete inhibited at 4 KGy, *Pseudomonas* spp no (21) , (42) and (43) completely inhibited at 5 KGy. It was found that the gamma irradiation doses decreased the percentages of the total number of viable bacterial cells.

Table (15) : Effect of gamma irradiation doses on some bacterial isolates

Bacterial Isolates	Dose (KGy)	No.of Cells	log no	Bacterial Isolates	Dose (KGy)	No. of cells	no log
E.coli (11)	0	$14\ 10^6$	7.1	*E.coli* (12)	0	22×10^6	9.2
	1	12×10^4	5		1	15×10^4	5.1
	2	3×10^2	2.5		2	17×10^2	3.2
	3	0	0		3	80	2
	4	0	0		4	0	0
	5	0	0		5	0	0
E.coli(13)	0	25×10^6	13.4	*Pseudomnas* spp(21)	0	33×10^7	8.5
	1	7×10^5	5.8		1	40×10^6	7.6
	2	2×10^2	2.3		2	55×10^5	6.7
	3	0	0		3	12×10^4	5
	4	0	0		4	70	1.8
	5	0	0		5	0	0
Proteus spp (34)	0	34×10^6	7.5	*Pseudomonas* spp(42)	0	23×10^7	8.4
	1	16×10^5	6.2		1	16×10^6	7.2
	2	11×10^3	4		2	9×10^4	4.9
	3	67	1.8		3	7×10^2	2.8
	4	0	0		4	89	1.9
	5	0	0		5	0	0
Pseudomonas spp(43)	0	19×10^7	8.3	*E.coli* (49)	0	34×10^6	7.5
	1	42×10^5	6.6		1	16×10^5	6.2
	2	7×10^3	3.8		2	14×10^3	4.1
	3	40	1.6		3	80	2
	4	0	0		4	0	0
	5	0	0		5	0	0

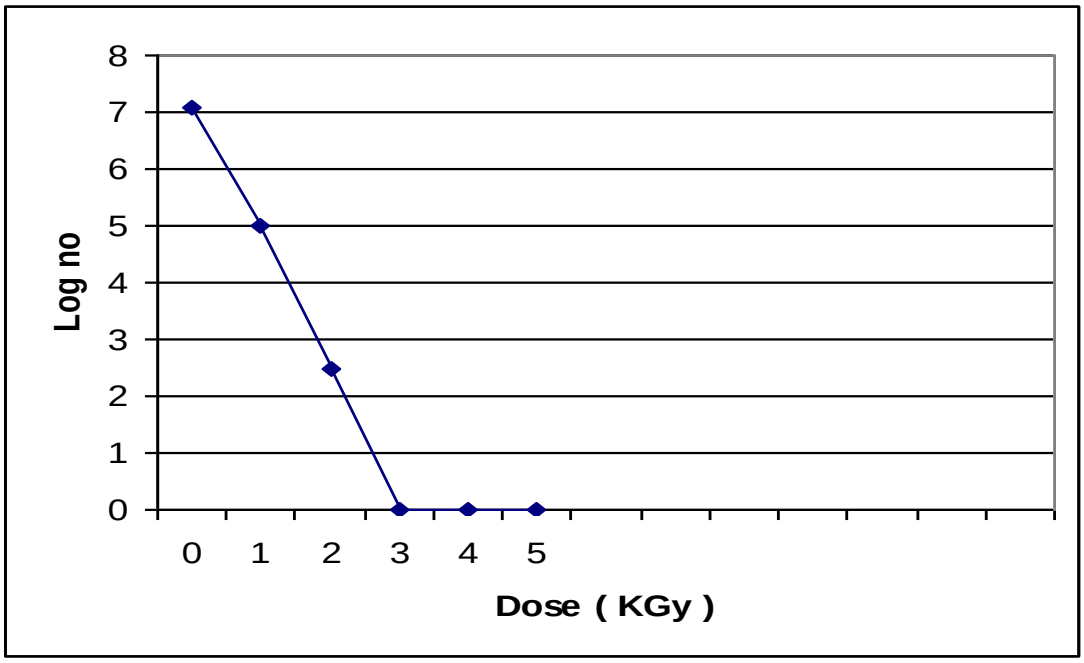


Figure (9): Dose survival curve of *E.coli* (11)viable cells after exposure to different doses of gamma rays (cobalt 60) .

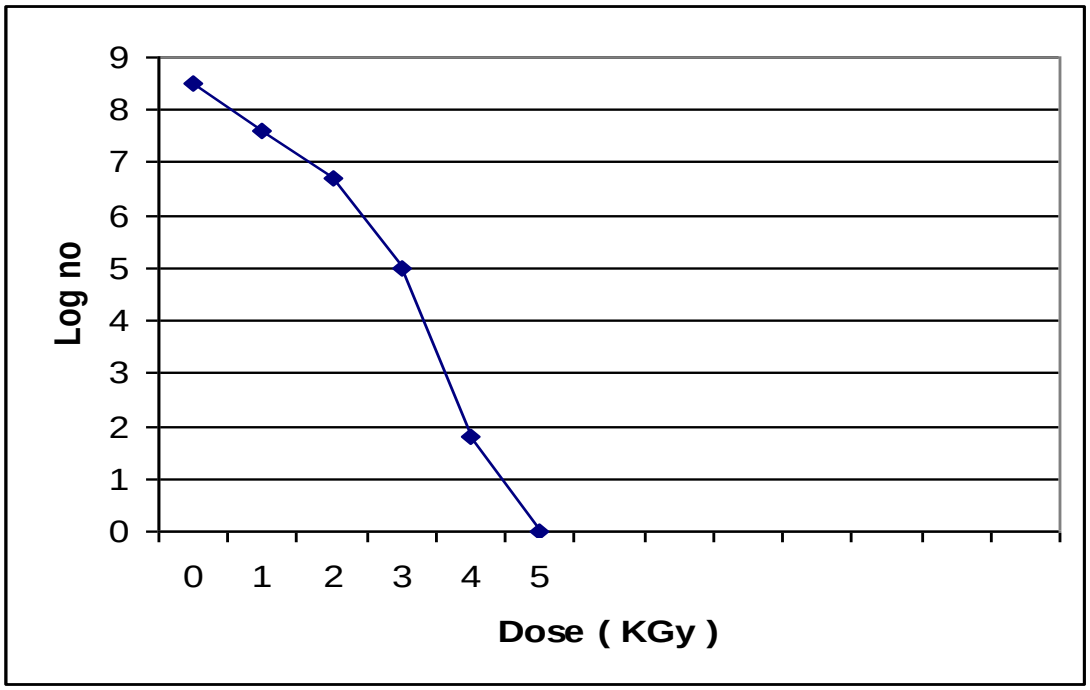


Figure (10): Dose survival curve of *Pseudomonas* spp(21) viable cells after exposure to different doses of gamma rays (cobalt 60) .

6.3. Antibacterial activity of X radiation :

In this experiment, the effect of X radiation after exposure of different doses (0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5,4, 4.5 and 5 kgy) for bacterial isolated, the results were shown in Table (16) and Figures (11) and (12). These results showed that the total viable counts for *E.coli* no (11), (12),(13) completely inhibited after 3 KGy of X rays. *Proteus* spp and *Pseudomonas* spp no (21), (42) were complete inhibited after 4 KGy of X rays while *Pseudomonas* spp no (43) was complete inhibited after 4.5 KGy of X ray. This means that the X radiation doses decreased the percentages of the total number of viable bacterial cells.

Table (16) : Effect of X rays on the growth of some bacterial isolates

Bacterial Isolates	Dose (KGy)	No of cells	Log . no	Bacterial isolates	Dose (KGy)	No of cells	no log
E.coli (11)	0	12×10^8	9.1	*E.coli* (12)	0	16×10^8	9.2
	0.5	10×10^7	8		0.5	8×10^7	7.9
	1	9×10^6	6.9		1	12×10^6	7
	1.5	2×10^4	4.3		1.5	7×10^3	3.8
	2	3×10^2	2.5		2	8×10^2	2.9
	2.5	50	1.7		2.5	2×10^2	2.3
	3	0	0		3	0	0
	3.5	0	0		3.5	0	0
	4	0	0		4	0	0
	4.5	0	0		4.5	0	0
	5	0	0		5	0	0
E.coli (13)	0	25×10^8	9.4	*Pseudomonas* spp (21)	0	34×10^8	9.5
	0.5	10×10^7	8		0.5	18×10^7	8.2
	1	9×10^4	4.9		1	11×10^6	7
	1.5	6×10^3	3.8		1.5	8×10^6	6.9
	2	4×10^2	2.6		2	9×10^5	6.9
	2.5	50	1.7		2.5	9×10^4	4.9
	3	0	0		3	3×10^3	3.9
	3.5	0	0		3.5	2×10^2	2.3
	4	0	0		4	0	0
	4.5	0	0		4.5	0	0
	5	0	0		5	0	0
Proteus spp (34)	0	26×10^7	8.4	*Pseudomonas* spp (42)	0	30×10^8	9.5
	0.5	15×10^6	7.2		0.5	18×10^7	8.2
	1	7×10^6	6.8		1	11×10^6	7
	1.5	12×10^5	6.1		1.5	8×10^6	6.9
	2	4×10^5	5.6		2	9×10^5	6.9
	2.5	8×10^4	4.9		2.5	9×10^4	4.9
	3	4×10^3	3.6		3	3×10^3	3.5
	3.5	2×10^2	2.3		3.5	2×10^2	2.3
	4	0	0		4	0	0
	4.5	0	0		4.5	0	0
	5	0	0		5	0	0
Pseudomonas spp (43)	0	33×10^7	8.5	*E.coli* (49)	0	15×10^8	9.2
	0.5	17×10^6	7.2		0.5	10×10^6	7
	1	10×10^5	6		1	12×10^5	6.8
	1.5	6×10^4	4.8		1.5	11×10^4	5.4
	2	13×10^3	4.1		2	2×10^3	3.3
	2.5	12×10^3	4		2.5	2×10^2	2.3
	3	10×10^2	3		3	0	0
	3.5	2×10^2	2.3		3.5	0	0
	4	130	2.1		4	0	0

	4.5	0	0		4.5	0	0
	5	0	0		5	0	0

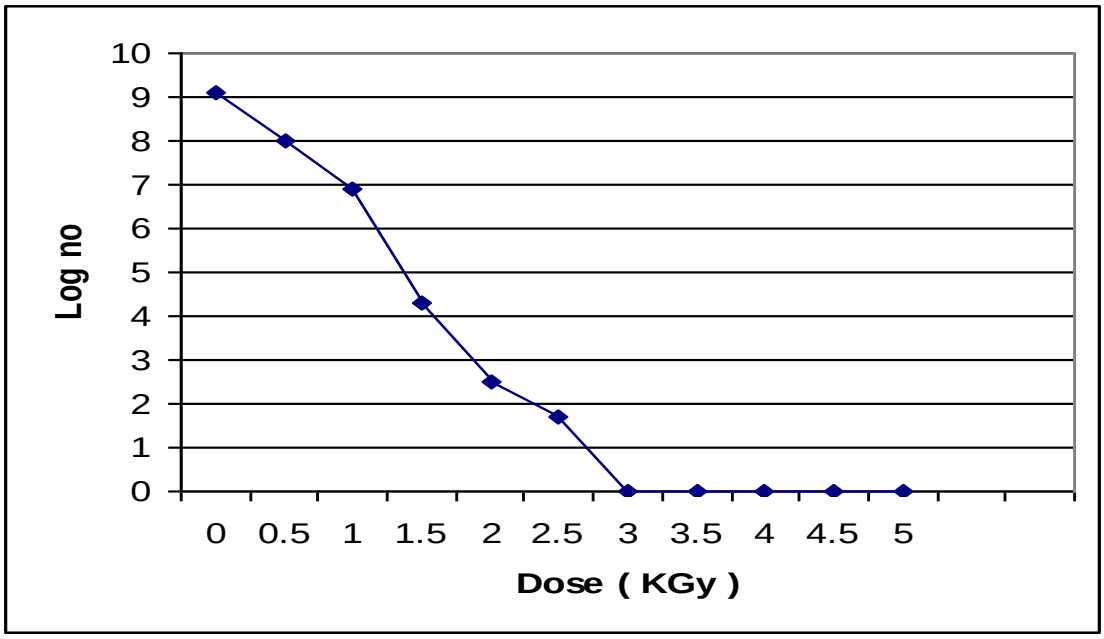


Figure (11) : Dose survival curve of *E.coli* (11) viable cells after exposure to different doses of X rays .

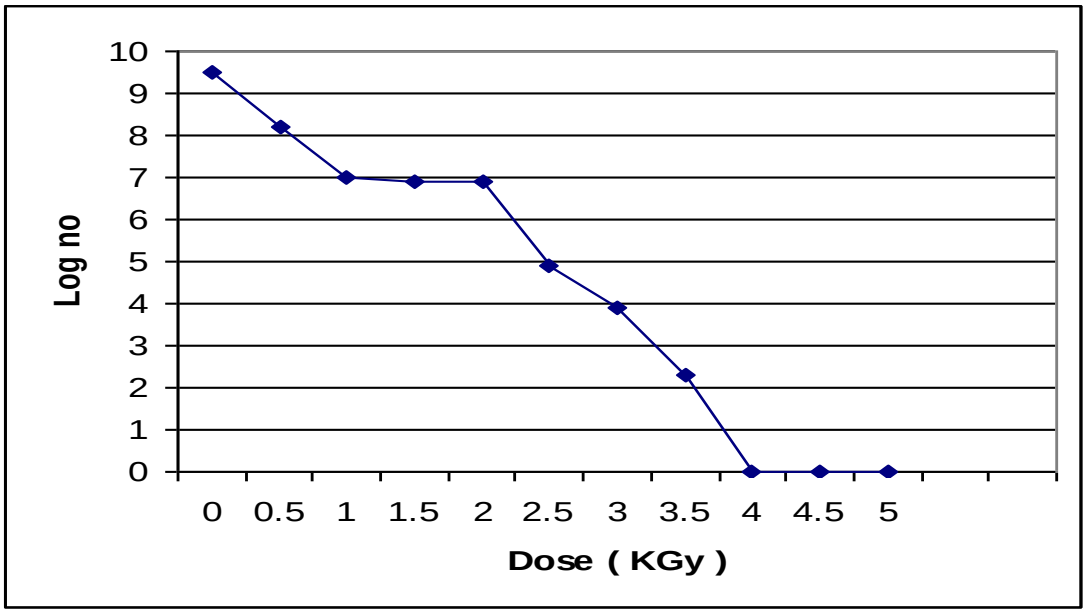


Figure (12) : Dose survival curve of *Pseudomonas* spp (21) viable cells after exposure to different doses of X rays .

7 - Effect of essential oils on the ultrastrcture of *Pseudomonas* spp using Transmission Electron Microscope (TEM)

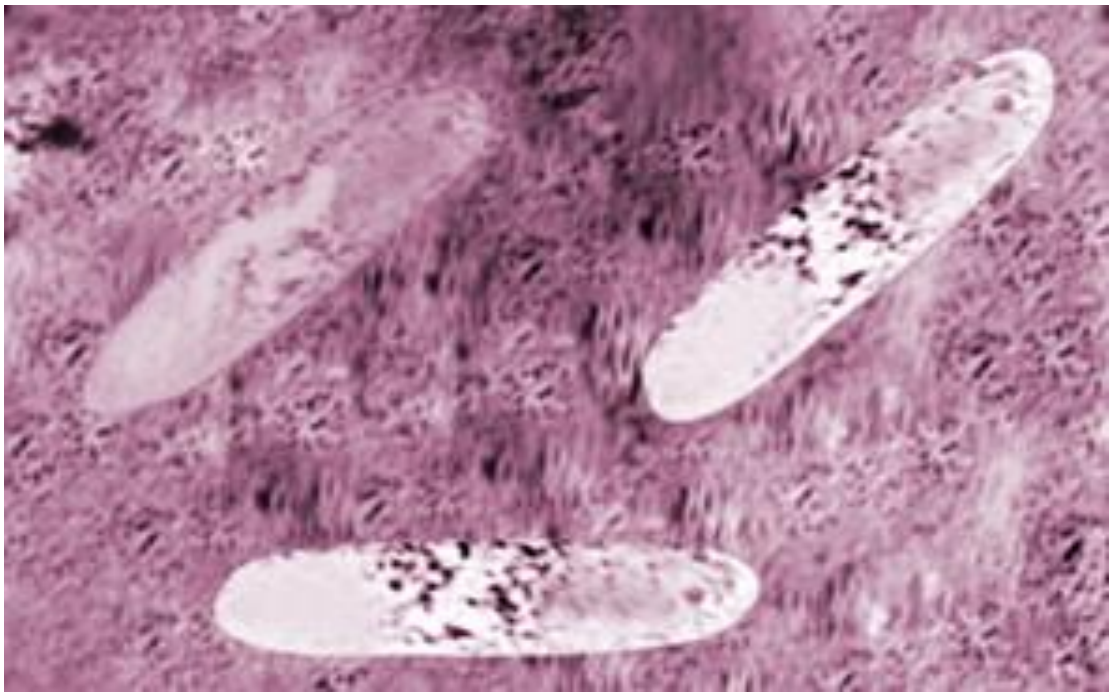
7.1. Effect of thyme oil

The thyme oil was the most effective against clinical *Pseudomonas* spp no (20) , and this oil was selected to study their effect on ultrastrcture of *Pseudomonas* spp to explain the sensitivity of this isolates to thyme oil and compared with non treated isolates .

The figures (16,17) clearly demonstrated that ultrastrcture of *Pseudomans* spp no (20) by Transmission Electron Microscope (TEM) after exposed to *Thymus vulgaris* (Thyme) oil had destructive effect on cell wall and cytoplasm substance compared with non treated (control) *Pseudomonas* spp .

Control (without oil)

Figure (13) :Transmission electron micrographs of *Pseudomonas* spp

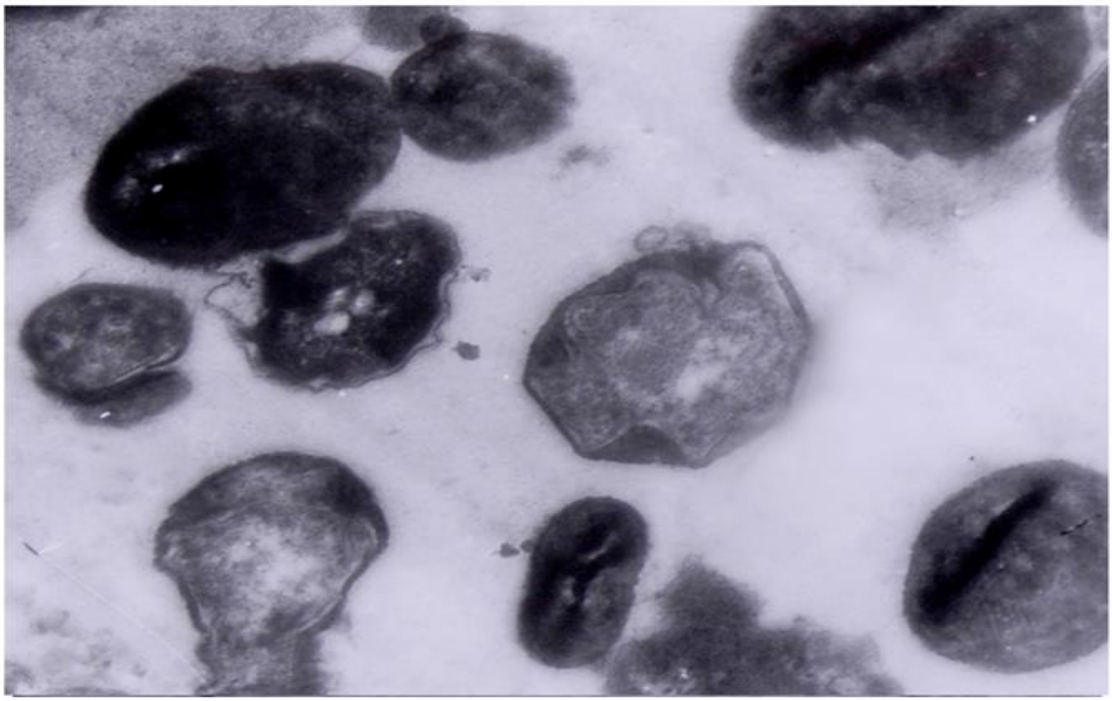


Treated(with oil)

Figure (14) : Transmission electron micrographs of *Pseudomonas* spp sensitive to *Thymus Vulgaris L* (Thyme) essential oil .

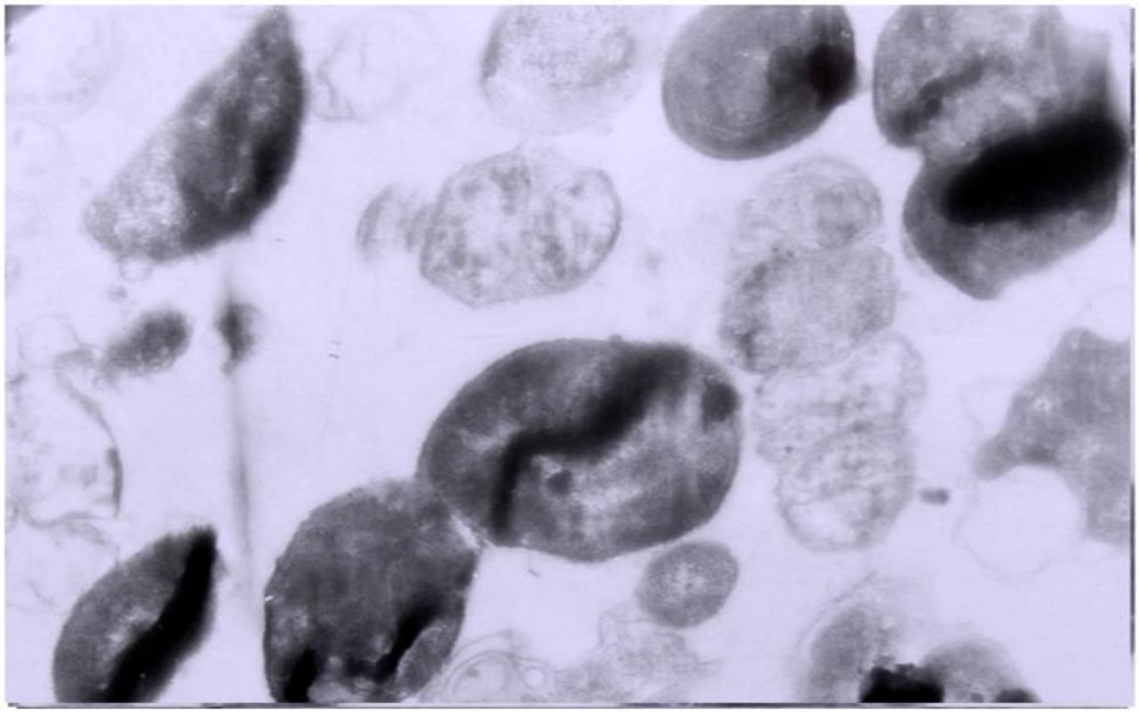
7.2. Effect of Jasmine oil

From antibacterial activity of different essential oils , it was found that *Jasmium gradiflorum L.*(Jasmine) essential oil had no effect on *Pseudomonas* spp, its effect was tested on the ultrastrcture of *Pseudomonas* spp no (24) using Transmission electron Microscope (TEM). These micrographs were illustrated in Figures (18 and 19). The micrograph of control (without essential oil) and treated (with essential oil) showed no obvious effect .



Control (without oil)

Figure (15) : Transmission electron micrographs of *Pseudomonas* spp (cross section)



Treated (oil)

Figure (16) : Transmission electron micrographs of *Pseudomonas* spp resistant to *Jasmium gradiflorum L.*(Jasmine) essential oil .

(cross section)