RESULTS

EXPERIMENTAL RESULTS

It has been found by many investigators that plasmids are the most important primitive oraganelles in bacteria carrying the majority of antibiotic resistant genes.

Therefore, the idea of the present investigation had been raised to clear the importance of plasmids in some *Ps.aeruginosa* isolates in antibiotic resistance and how it can be reduced either through plasmid curing or genetic alteration. Recently, by changing the nucleotide sequences of the site promotor or leader can change the level of resistance (Alexieva *et al.*, 1988).

In the present study, five isolates of *Pseudomonas aeruginosa* were isolated from the urine of 20 patients with chronic urinary tract infection and 20 patients with cancer bladder. Two isolates 1 & 2 from the former and three isolates 3, 4, 5 from the latter.

All isolates were Gram negative, actively motile bacilli. On the solid media they revealed the following characteristics :-

-All isolates showed different colonies especially large and small ones, with dark greyish center and

irregular translucent edges, with odour of trimethylamine.

-Most isolates had coloured pigments after incubation at 37°C for 24 hours .

-All were oxidase positive rapidly producing purple colour with 0.5% NN-dimethylphenylene diamine monohydrochloride.

-All formed slime and surface pellicle when grown in nutrient broth.

-All liquefied gelatin.

-All fermented glucose oxidatively with acid production.

-All grew readily at 42°C and optimally at 37°C.

-All oxidized gluconate to 2 - ketogluconate which formed orange precipitate with Bendict's reagent.

-All utilized malonate.

Pseudomonas aeruginosa Sensitivity to Amikacin :-

Table(1) presents the mean inhibition zones for each isolate in five replicates/mm using antibiotic discs each containing 30 μg and their standard errors.

Table(1) shows that the sensitivities of the five Pseudomonas isolates to amikacin are nearly equal. They all are sensitive according to Lennette *et al*. (1974) who indicates that the zone of inhibition should be less than 14 mm to be considered as resistant.

Table (1) : Mean inhibition zones of Pseudomonas aeruginosa isolates obtained with 30 µg amikacin /mm.

Isolate	Replicates						
	1	2	3	4	5	Mean	S.E
1	13	14	16	17	15	15	± 0.70
2	18	16	16	15	14	16	± 0.67
3	14	18	15	16	15	16	± 0.70
4	15	16	14	17	13	15	± 0.70
5	18	19	17	15	16	17	± 0.70

Table (2) : Mean inhibition zones of Pseudomonas aeruginosa isolates obtained with 10 µg tobramycin/mm.

Isolate	Replicates						
	1	2	3	4	5	Mean	S.E
1	2	6	5	3	4	4	± 0.70
2	14	17	13	15	16	15	± 0.70
3	13	12	13	14	13	13	± 0.31
4	12	16	14	13	15	14	± 0.70
5	11	13	12	10	14	12	± 0.70

Pseudomonas aeruginosa Sensitivity to Tobramycin :

Table(2) illustrates the mean inhibition zones for each isolate in five replicates/mm using antibiotic discs each containing 10 μg and their standard errors.

Table(2) shows that the sensitivities of the five Pseudomonas isolates to tobramycin are different. The most resistant isolate was no.I according to Lennette *et al.* (1974) who indicates that zone of inhibition should be less than 12mm to be considered as resistant.

Pseudomonas aeruginosa Sensitivity to Kanamycin:-

Table(3) presents the mean inhibition zones for each isolate in five replicates/mm using antibiotic discs each containing 30 μg and their standard errors.

Table(3) shows that the sensitivities of the five Pseudomonas isolates to kanamycin are different. The most resistant isolate was no. I according to Lennette *et al.* (1974) who indicates that the zone of inhibition should be less than 13 mm to be considered as resistant.

Pseudomonas aeruginosa Sensitivity to Streptomycin:-

Table(4) illustrates the mean inhibition zones for each isolate in five replicates/mm using antibiotic discs each containing 10 μg and their standard errors.

Table (3) : Mean inhibition zones of Pseudomonas aeruginosa isolates obtained with 30 µg kanamycin/mm.

Isolate	Replicates						
	1	2	3	4	5	Mean	S.E
1	7	3	4	6	5	5	± 1.22
2	14	20	17	15	19	17	± 1.14
3	4	12	7	9	8	8	± 1.30
4	5	7	4	8	6	6	± 0.70
5	7	13	10	14	6	10	± 1.58

Table (4) : Mean inhibition zones of Pseudomonas aeruginosa isolates obtained with 10 µg streptomycin/mm.

Isolate	Replicates						
	1	2	3	4	5	Mean	S.E
1	2	3	4	1	5	3	± 0.70
2	10	16	15	13	11	13	± 1.14
3	9	11	10	12	8	10	± 0.70
4	2	6	3	5	4	4	± 0.70
5	4	8	5	6	7	6	± 0.70

Table(4) shows that the sensitivities of the five Pseudomonas isolates to streptomycin are different. The most resistant isolate was no. I according to Lennette *et al.s* (1974) who indicates that the zone of inhibition should be less than 11 mm to be considered as resistant.

Pseudomonas aeruginosa Sensitivity to Gentamicin :-

Table(5) presents the mean inhibition zones for each isolate in five replicates/mm using antibiotic discs each containing 10 µg and their standard errors.

Table(5) shows that the sensitivities of the five Pseudomonas isolates to gentamicin are different. The most resistant isolate was no. I according to Lennette *et al.* (1974) who indicates that the zone of inhibition should be less than 12 to be considered as resistant.

Antibiotics resistance markers for Pseudomonas aeruginosa isolates:-

From the previous five tables antibiotic resistance markers can be determined for each of the five Pseudomonas isolates.

Table (5) : Mean inhibition zones of Pseudomonas aeruginosa isolates obtained with 10 µg gentamicin/mm.

Isolate	Replicates						
	1	2	3	4	5	Mean	S.E
1	3	9	8	6	4	6	± 1.14
2	8	12	10	11	9	10	± 0.70
3	11	13	9	12	15	12	± 1.00
4	6	10	9	8	7	8	± 0.70
5	10	12	8	14	6	10	± 1.41

Table (6) : Antibiotic resistance markers for Pseudomonas aeruginosa isolates

Isolate	Antibiotic resistance pattern
1	Tm, Km, Sm, Gm
2	Gm
3	Km, Sm
4	Km, Sm, Gm
5	Km, Sm, Gm

Km = kanamycin

Gm = gentamicin

Sm = streptomycin

Tm = tobramycin

Tables(6) showed that isolates no. I is resistant to the four antibiotics; tobramycin, kanamycin, streptomycin and gentamicin. Isolates no. 4 and 5 are resistant to three antibiotics;kanamycin, streptomycin and gentamicin. Isolate no. 3 is resistant to antibiotics; kanamycin and streptomycin while the isolate no. 2 is resistant only to gentamicin.

The Determination of the Different Antibiotics Minimum Inhibitory Concentrations (MIC) for the Pseudomonas isolates :-

Different concentrations from each antibiotic were used in solid nutrient agar medium to determine the MIC.

Table(7a) presents the concentration of each antibiotic and the luxurious growth of the isolate no, I.

Table (7a) showed that the lowest MIC for the isolate no. I was that of Am (50 µg/ml) followed by Tm 400 µg/ml and Gm 400 µg/ml while those for Km and Sm reached > 400 µg/ml.

Table (7b) illustrates the concentration of each antibiotic and the luxurious growth of isolate no.2. It showed that MIC for isolate 2 to the antibiotics;Am,

Table (7a) : Pseudomonas aeruginosa MIC for different antibiotics (Isolate 1)

Conc. µg/ml	No. of colonies survived				
	Antibiotics Am	Tm	Km	Sm	Gm
0	>10,000	>10,000	>10,000	>10,000	>10,000
3.125	>1000	>10,000	>10,000	>10,000	>10,000
6.25	>1000	>10,000	>10,000	>10,000	>10,000
12.5	>500	>1000	>10,000	>10,000	>10,000
25	>100	>1000	>10,000	>10,000	>1000
50	0	>1000	>10,000	>10,000	>500
100	0	>500	>1000	>1000	>100
200	0	>100	>500	>500	>100
400	0	0	>100	>100	0

Table (7b) : Pseudomonas aeruginosa MIC for different antibiotics (Isolate 2)

Conc. µg/ml	No. of colonies survived				
	Antibiotics Am	Tm	Km	Sm	Gm
0	>10,000	>10,000	>10,000	>10,000	>10,000
3.125	>500	>1000	>10,000	>1000	>10,000
6.25	>100	>500	>1000	>500	>1000
12.5	>100	>100	>500	>100	>1000
25	0	>100	>100	>100	>500
50	0	0	>100	0	>100
100	0	0	0	0	0
200	0	0	0	0	0
400	0	0	0	0	0

Am = amikacin Km = kanamycin Gm = gentamicin

Sm = streptomycin Tm = tobramycin

Tm, Km, Sm and Gm are: 25, 50, 100, 50, and 100 µg/ml. respectively.

Table (7c) presents the same for isolate 3. It showed that MIC for this isolate to the same antibiotics are :12.5, 100, 400, >400 and 50 µg/ml, respectively.

Table(7d) illustrates the same for isolate no.4. It showed that MIC for this isolate to the same antibiotics are; 25, 100, >400, >400 and 50 µg/ml respectively.

Table (7e) presents the same for isolate 5. It showed that MIC for this isolate to the same antibiotics are 12.5, 25, 100, 200 and 100 µg/ml, respectively.

Table (7c) : Pseudomonas aeruginosa MIC for different antibiotics (Isolate 3)

Conc. µg/ml	No. of colonies survived				
	Antibiotics Am	Tm	Km	Sm	Gm
0	>10,000	>10,000	>10,000	>10,000	>10,000
3.125	>100	>10,000	>10,000	>10,000	>1000
6.25	>100	>1000	>10,000	>1000	>500
12.5	0	>500	>10,000	>1000	>100
25	0	>100	>1000	>1000	>100
50	0	>100	>1000	>500	0
100	0	0	>500	>500	0
200	0	0	>100	>500	0
400	0	0	0	>100	0

Table (7d) : Pseudomonas aeruginosa MIC for different antibiotics (Isolate 4)

Conc. µg/ml	No. of colonies survived				
	Antibiotics Am	Tm	Km	Sm	Gm
0	>10,000	>10,000	>10,000	>10,000	>10,000
3.125	>500	>1000	>10,000	>10,000	>1000
6.25	>500	>500	>1000	>1000	>500
12.5	>100	>500	>1000	>1000	>100
25	0	>100	>500	>1000	>100
50	0	>100	>500	>500	0
100	0	0	>100	>500	0
200	0	0	>100	>500	0
400	0	0	>100	>100	0

Am = amikacin Km = kanamycin Gm = gentamicin

Sm = streptomycin Tm = tobramycin

Table (7e) : Pseudomonas aeruginosa MIC for different antibiotics (Isolate 5)

Conc. µg/ml	No. of colonies survived				
	Antibiotics Am	Tm	Km	Sm	Gm
0	>10,000	>10,000	>10,000	>10,000	>10,000
3.125	>500	>1000	>1000	>10,000	>1000
6.25	>100	>500	>500	>1000	>1000
12.5	0	>100	>100	>1000	>500
25	0	0	>100	>500	>500
50	0	0	>100	>500	>100
100	0	0	0	>100	0
200	0	0	0	0	0
400	0	0	0	0	0

Am = amikacin Km = kanamycin Gm = gentamicin

Sm = streptomycin Tm = tobramycin

The Mode of Inheritance of Antibiotic Resistance:-

To determine the mode of inheritance of each antibiotic resistance for each isolate the method proposed by Ali *et al*.(1985 a & b) was applied.

Twenty five single colonies from each isolate were tested for their level of resistance on each of the antibiotic which it resists.

Table(8) presents the growth behavior of 25 single colonies from isolate I on the concentrations of; 0, 25, 50, 100 and 200 µg/ml Sm, Km, Gm and Tm. respectively.

Table(8) showed great variation for isolate no. I single colonies resistance to; Sm, Km, Gm and Tm.This indicates that streptomycin, kanamycin, gentamicin and tobramycin resistant genes in this isolate are located on plasmids which spontaneously eliminated from some single colonies during growth.

Fig (1 & 2) diagrams showing single colonies resistance levels to different antibiotics.

Table (9) presents gentamicin resistance levels for 25 single colonies from isolate no. 2. All 25 colonies showed similar resistance levels indicating their chromosomal location.

Table (10) illustrates Km and Sm resistance levels for 25 single colonies for isolate no.3. It showed that the single colonies of isolate 3 resist different levels of the antibiotics; Km and Sm. This indicates plasmid mode of inheritance for antibiotics resistance. Moreover, complete agreement can be noticed for the resistance levels of each single colony to both antibiotics. This latter phenomenon might indicates the existence of Km and Sm resistant genes in the same plasmid in this isolate.

Table (11) presents Km, Sm, and Gm resistance levels for 25 single colonies for isolate no.4. It showed that the single colonies of isolate no.4 resist different levels of the three antibiotics indicating plasmid mode of inheritance. Great agreement for resistance levels of each of the single colonies to the three different antibiotics can be noticed. This might indicate the existence of the genes responsible for these different antibiotics resistance in the same plasmids.

Isolate no.5 resist the same three antibiotics; Km, Sm and Gm (Table 6). This might indicate similar behavior to that of isolate no.4.

Table (8) Isolate I single colonies examination on different antibiotics concentrations

Colony No.	Control 0	Sn conc. µg/ml				Kn conc. µg/ml				Gn conc. µg/ml				Tn conc. µg/ml			
		25	50	100	200	25	50	100	200	25	50	100	200	25	50	100	200
1	+	+	+	-	-	-	-	-	-	+	+	+	-	+	+	+	-
2	+	+	+	+	-	+	+	+	-	-	-	-	-	-	-	-	-
3	+	-	-	-	-	+	-	-	-	+	+	+	+	+	+	+	+
4	+	+	-	-	-	+	+	-	-	+	-	-	-	-	-	-	-
5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
6	+	+	+	-	-	+	+	+	-	+	+	-	-	+	+	-	-
7	+	+	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-
8	+	+	+	+	-	+	+	+	-	+	+	+	+	+	+	-	-
9	+	+	+	-	-	-	+	+	-	+	+	+	-	+	-	-	-
10	+	+	+	-	-	-	+	-	-	+	+	+	+	+	+	+	+
11	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-
12	+	+	+	+	-	+	+	+	-	+	-	-	-	+	+	-	-
13	+	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-
14	+	-	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-
15	+	+	+	+	-	+	+	+	-	+	-	-	-	+	+	-	-
16	+	+	+	-	-	+	+	-	-	+	-	-	-	+	+	-	-
17	+	+	-	-	-	+	-	-	-	+	-	-	-	+	+	-	+
18	+	+	+	-	-	+	-	-	-	-	-	-	-	+	+	-	-
19	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	-
20	+	+	-	-	-	+	-	-	-	+	+	-	-	+	+	-	-
21	+	+	+	+	+	+	+	+	-	+	+	-	-	+	+	-	-
22	+	+	-	-	-	+	-	-	-	+	+	-	-	+	-	-	-
23	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-
24	+	-	-	-	-	+	-	-	-	-	-	-	-	+	+	+	-
25	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
‡	+	21	17	10	6	22	16	12	5	19	12	8	5	20	17	6	4

‡ : No. of Resistant colonies

Kn : kanamycin Sn : streptomycin Gn : gentamicin Tn : tobramycin

Fig. (1) Diagram showing single colonies
Resistance levels to different antibiotics.

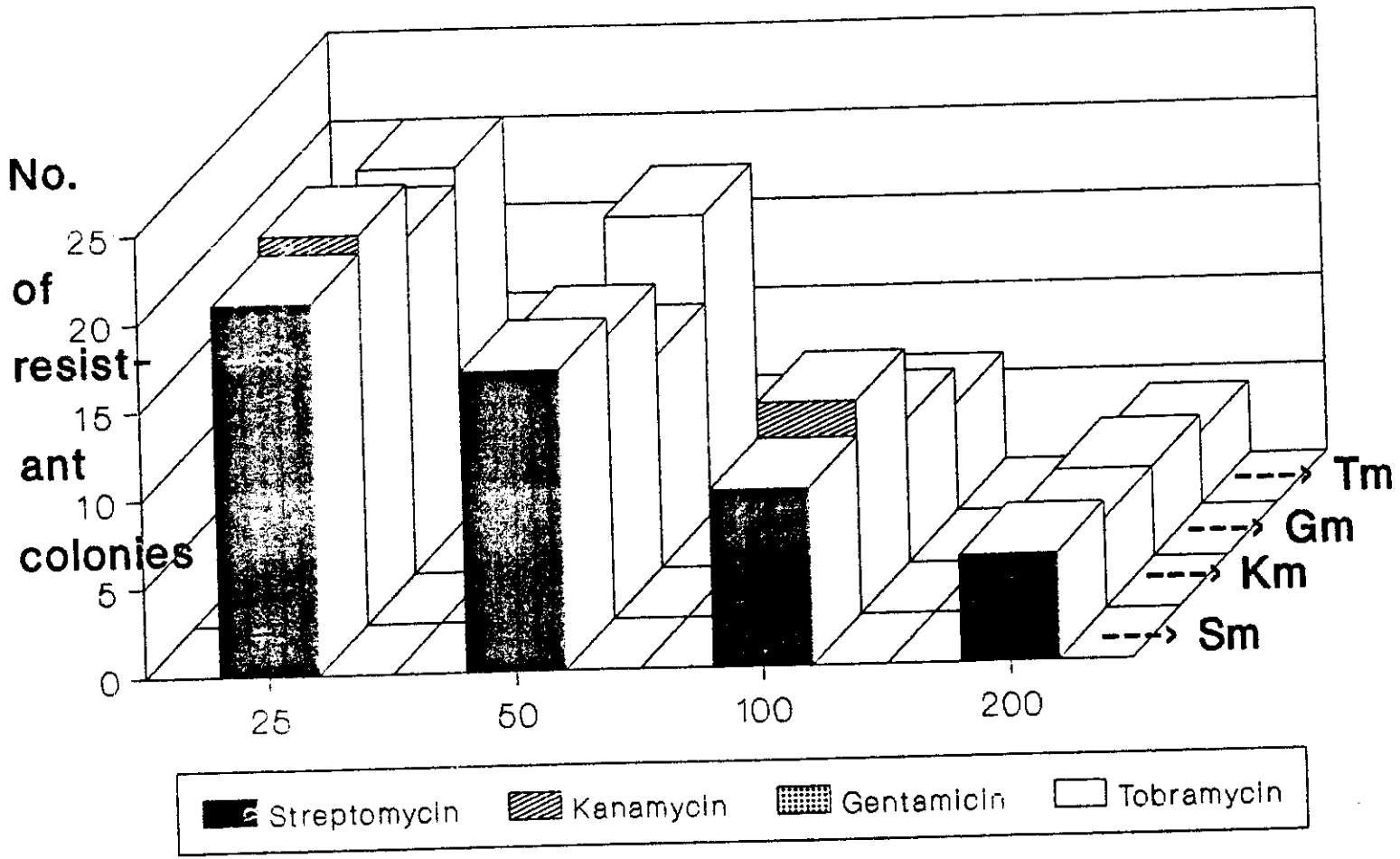


Fig. (2) Diagram showing single colonies
Resistance levels to different antibiotics.

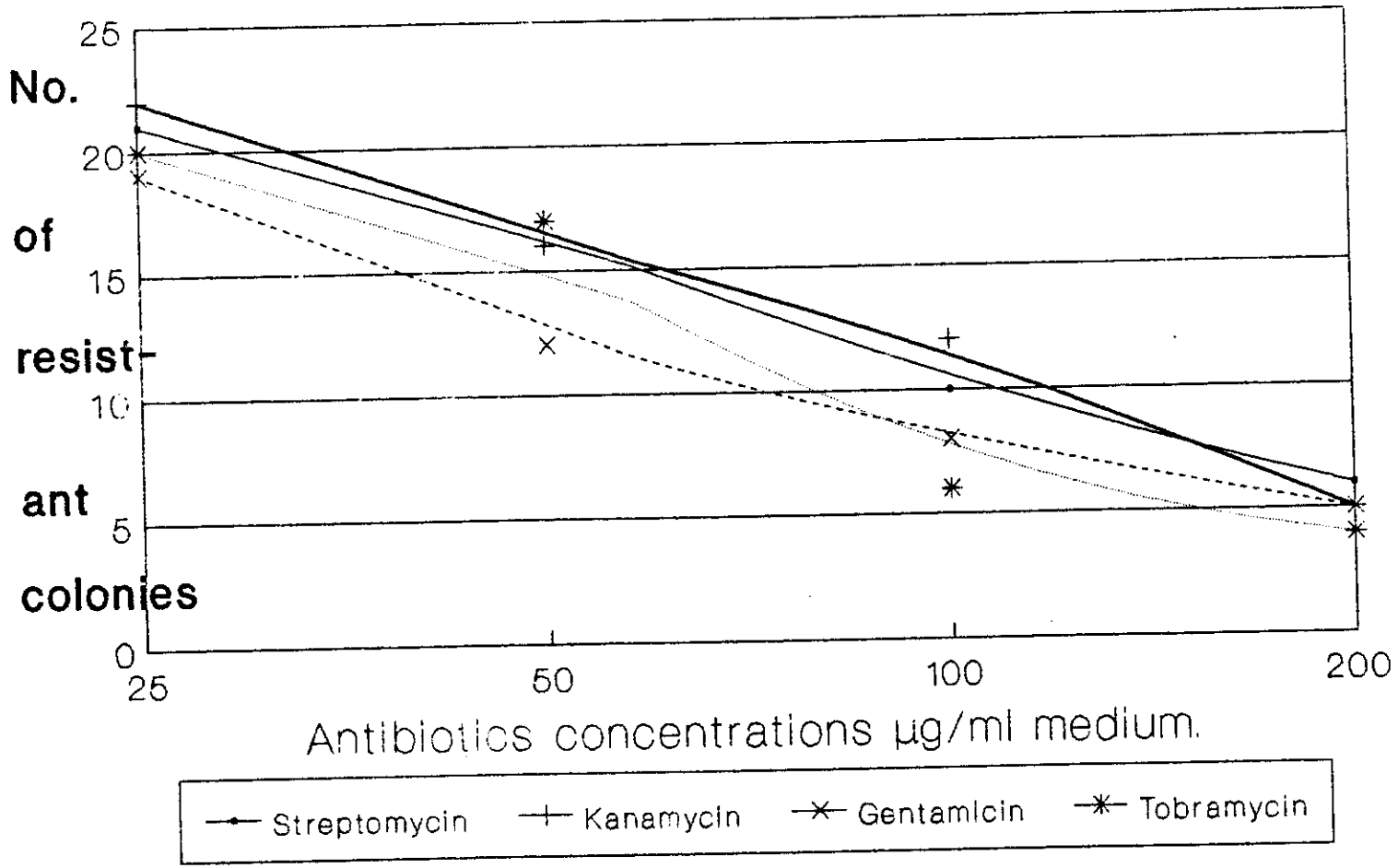


Table (9): Isolate 2 single colonies examination on different Gentamicin concentrations.

Colony No.	Control 0	Gentamincin Concentration µg/ml 6.25	12.5	25	50
1	+	+	+	+	+
2	+	+	+	+	+
3	+	+	+	+	+
4	+	+	+	+	+
5	+	+	+	+	+
6	+	+	+	+	+
7	+	+	+	+	+
8	+	+	+	+	+
9	+	+	+	+	+
10	+	+	+	+	+
11	+	+	+	+	+
12	+	+	+	+	+
13	+	+	+	+	+
14	+	+	+	+	+
15	+	+	+	+	+
16	+	+	+	+	+
17	+	+	+	+	+
18	+	+	+	+	+
19	+	+	+	+	+
20	+	+	+	+	+
21	+	+	+	+	+
22	+	+	+	+	+
23	+	+	+	+	+
24	+	+	+	+	+
25	+	+	+	+	+
*	+	25	25	25	25

* = No. of Resistant Colonies

Table (10) Isolate No. 3 single colonies examination on different Km and Sm concentrations.

Colony No.	Control 0	Km conc.µg/ml				Sm conc.µg/ml			
		25	50	100	200	25	50	100	200
1	+	+	+	+	−	+	+	+	−
2	+	+	+	+	+	+	+	+	+
3	+	+	−	−	−	+	−	−	−
4	+	+	+	−	−	+	+	−	−
5	+	+	+	+	−	+	+	+	−
6	+	+	+	+	+	+	+	+	+
7	+	+	−	−	−	+	−	−	−
8	+	−	−	−	−	−	−	−	−
9	+	+	+	+	−	+	+	+	−
10	+	+	+	−	−	+	+	−	−
11	+	+	−	−	−	+	−	−	−
12	+	+	+	−	−	+	+	−	−
13	+	−	−	−	−	−	−	−	−
14	+	+	+	+	−	+	+	+	−
15	+	+	+	−	−	+	+	−	−
16	+	+	−	−	−	+	−	−	−
17	+	+	+	−	−	+	+	−	−
18	+	+	+	+	+	+	+	+	+
19	+	+	+	+	−	+	+	+	−
20	+	+	+	−	−	+	+	−	−
21	+	−	−	−	−	−	−	−	−
22	+	+	+	−	−	+	+	−	−
23	+	+	+	+	+	+	+	+	+
24	+	+	−	−	−	+	−	−	−
25	+	+	+	−	−	+	+	−	−
*	+	22	17	9	4	22	17	9	4

* = No. of Resistant Colonies

Km = kanamycin

Sm = streptomycin

Table (11) Isolate No. 4 single colonies examination on different Km, Sm, and Gm concentrations.

Colony No.	Control	Sm conc. µg/ml				Km conc. µg/ml				Gm conc. µg/ml			
		25	50	100	200	25	50	100	200	25	50	100	200
1	+	+	+	-	-	+	+	-	-	+	-	-	-
2	+	+	+	+	-	+	+	+	-	+	+	+	-
3	+	-	-	-	-	-	-	-	-	-	-	-	-
4	+	+	-	-	-	+	-	-	-	+	-	-	-
5	+	+	+	-	-	+	+	-	-	+	+	-	-
6	+	-	-	-	-	-	-	-	-	-	-	-	-
7	+	+	+	+	-	+	+	+	-	+	+	+	-
8	+	+	+	-	-	+	+	-	-	+	+	-	-
9	+	+	+	+	-	+	+	+	-	+	+	+	-
10	+	+	+	-	-	+	+	-	-	+	+	-	-
11	+	+	-	-	-	+	-	-	-	+	-	-	-
12	+	+	+	+	-	+	+	+	-	+	+	+	-
13	+	+	+	-	-	+	+	-	-	+	+	-	-
14	+	+	+	-	-	+	+	-	-	+	+	-	-
15	+	+	-	-	-	+	+	-	-	+	+	-	-
16	+	+	+	+	-	+	+	+	-	+	+	+	-
17	+	-	-	-	-	-	-	-	-	-	-	-	-
18	+	+	+	+	-	+	+	+	-	+	+	+	-
19	+	+	+	+	+	+	+	+	+	+	+	+	+
20	+	+	+	-	-	+	+	-	-	+	+	-	-
21	+	+	+	-	-	+	+	-	-	+	+	-	-
22	+	+	+	+	+	+	+	+	+	+	+	+	+
23	+	+	+	-	-	+	+	+	-	+	+	+	-
24	+	+	+	+	-	+	+	+	-	+	+	+	-
25	+	+	+	-	-	+	+	-	-	+	+	-	-
♯	+	22	19	9	2	22	19	9	2	22	18	10	2

♯ = No. of Resistant Colonies

Sm = streptomycin Gm = gentamicin Km = kanamycin

Transformation Experiments:-

1- Gram negative bacilli (*E.Coli*, K12)

Plasmids DNA was isolated from *P.aeruginosa* isolate no.I which resist the four antibiotics; Sm, Km, Gm and Tm by the method mentioned previously in Materials and Methods.

The isolated plasmids DNA was used to transform *E.coli*, K12 which is sensitive to all four antibiotics. Sample of 0.5 ml plasmid DNA preparation containing 176 µg DNA/ml were used to transform 0.5 samples of *E.coli*, K12 suspension containing 10^5/ml competents bacterial cells.

Table (12) presents the number of competents *E.coli*, K12, the number and percentages of transformation to each of the four antibiotics; Sm, Km, Gm and Tm using 10 µg of each antibiotic/ml medium. It showed that the percentages of transformants ranged from 1 - 1.75 %.

Twenty five transformants for each antibiotic were tested for their resistant levels by streaking on nutrient agar plates divided to 25 squares with a grid supplemented with; 0, 50, 75, 100, 125, 150, 175 or 200 µg/ml medium from each antibiotic.

Table (12): The number and percentages of E.coli, K_{12} transformants resist 10 µg of each of the four antibiotics/ml medium.

Antibiotic	No. of Competent cells/ml	No. of transformants	%
Sm	10^5	1600	1.6(
Km	10^5	1750	1.7
Gm	10^5	1480	1.4(
Tm	10^5	1000	1.0(

Km = kanamycin

Sm = streptomycin

Gm = gentamicin

Tm = tobramycin

Table (13): The number of transformants and percentages which resist, 0, 50, 75, 100, 125, 150, 175 and 200 µg Sn, Km, Gn, Tn/ml medium

Antibiotic conc. µg/ml	0	50	75	100	125	150	175	200
Sn: No. of trans.	25	20	18	15	12	10	10	8
%	100	80	72	60	48	40	40	32
Km: No. of trans.	25	18	14	14	10	10	6	6
%	100	72	56	56	40	40	24	24
Gn: No. of trans.	25	15	10	7	5	2	2	1
%	100	60	40	28	20	8	8	4
Tn: No. of trans..	25	12	8	5	3	1	1	0
%	100	48	32	20	12	4	4	0

Km : kanamycin

Sn : streptomycin

Gn : gentamicin

Tn : tobramycin

Fig (3) Histogram showing the frequency of resistance transformants to different levels of the four antibiotics

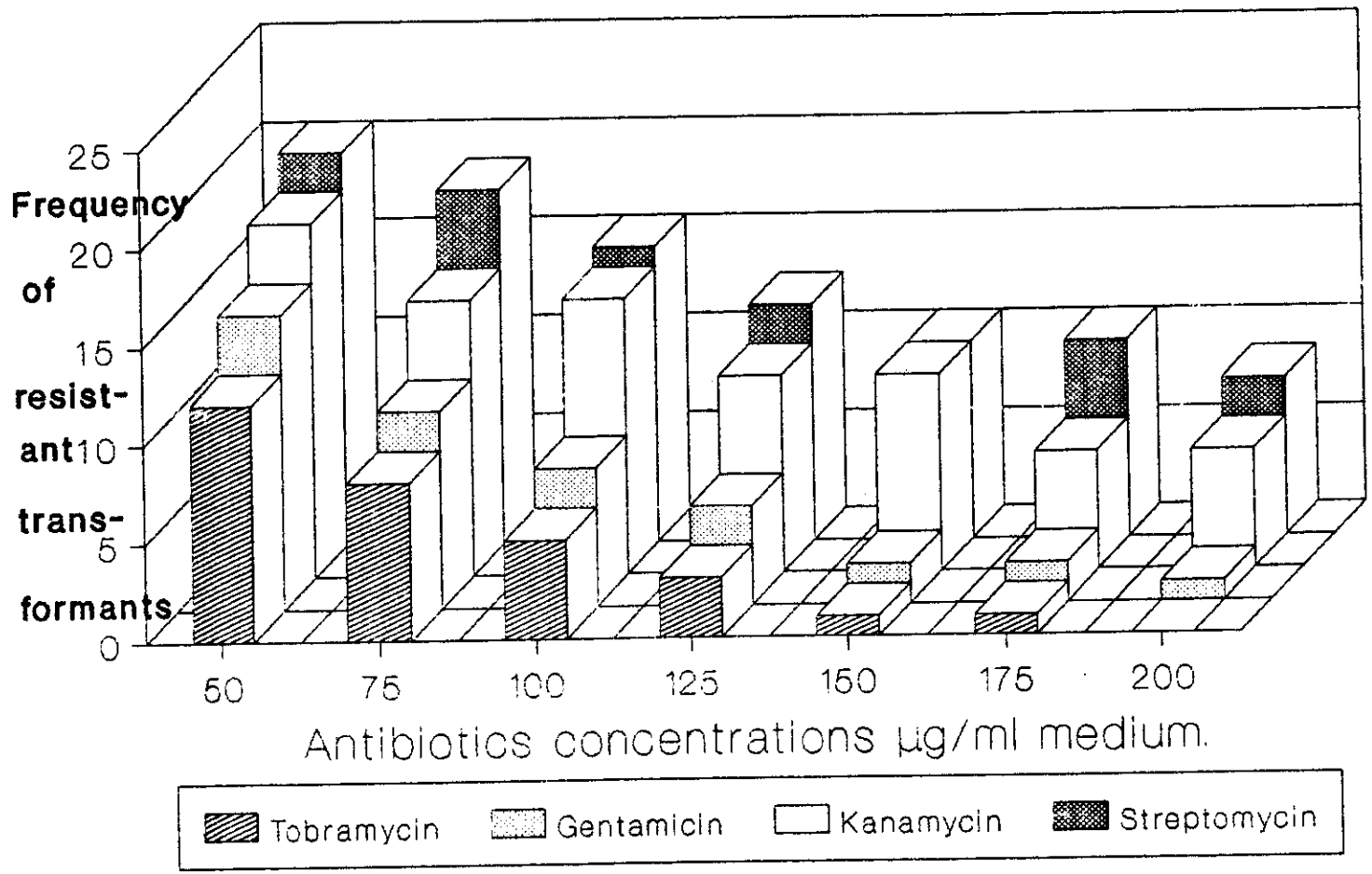


Table (13) illustrates the numbers and percentages of transformants which resist each antibiotic concentration.

Table (13) shows that streptomycin resistant *E.coli*, K12 transformants showed the highest levels of resistance followed by those to; Km, Gm and Tm. respectively.

Fig (3) is a histogram showing the frequency of resistant transformants to different levels of the four antibiotics.

2- Gram positive bacteria:-

However, transformation experiments had been done to the gram positive bacteria; *Staph.aureus* and *Bacillus subtilis* in the same manner. No antibiotic resistant transformants could be obtained from either, although many attempts had been done.

These results of transformation with plasmid DNA indicate the specificity of plasmids in gram negative and gram positive bacteria which are in agreement with those found by (Ehrlich, 1977; Wilson *et al*.,1981) and Horinouchi & Weisblum, 1982).

The Estimation of Plasmid Numbers:-

1- Kanamycin:-

To estimate the number of plasmids acquired by

E.coli, K12 transformants which resist Km, each transformants was tested in different levels of Km concentrations, i.e 10, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190 and 200 µg/ml medium.

Table (14) presents the growth behavior of 25 Km resistant *E.coli*,K12 transformants on different Km concentrations. It shows great variability in the levels of resistance. Seven transformants resist only 10 µg/ml medium, 5 transformants resist only 50 µg/ml medium, 5 transformants resist 100 µg/ml medium, 5 transformants resist 150 µg/ml medium and 3 transformants resist 200 µg km/ml medium.

These results indicate that the lowest resistance which can be acquired with a single plasmid is 10 µg km/ml medium. The level of 50 µg km resistant can be obtained from double dose of plasmid as no 20, 30 or 40 µg km resistant could be obtained. This increase in the double dose of plasmids in km resistant level can be due to gene interaction and gene regulatory mechanisms (Mongkolsuk *et al.*, 1984 ;Ambulos *et al.* 1985 a & b Ambulos *et al.*, 1986; Duvall & Lovett, 1986 and Duvall *et al.*, 1987). On the same basis one can estimate 3 plasmids for the level of 100 µg km/ ml, 4 plasmids for

the level of 150 μg km/ml and 5 plasmids for the level of 200 μg km/ml.

Fig (4) Diagram showing *E. coli*, k12 transformants resistance level to kanamycin.

2- Streptomycin :-

Table(15) presents the growth behavior of 25 Sm resistant *E. coli*, k12 transformants on different Sm. concentrations. It shows great variability in the levels of resistance. Five transformants resist 50 μg Sm/ml medium, 6 transformants resist 100 μg Sm/ml medium, 6 transformants resist 150 μg Sm/ml and 8 transformants resist 200 μg Sm/ml medium. These results indicate that each plasmid introduce 50 μg Sm resistant level. Therefore each transformant which resist 50 μg Sm. contains only one plasmid. Each of these which resist 100 μg Sm contains two plasmids, while each of those resist either 150 or 200 μg Sm, contains 3 or 4 plasmids respectively. No effects of gene interactions or regulatory mechanisms can be noticed.

Fig (4) diagram showing E. coli, k12 transformants resistance level streptomycin.

Table (14) The growth behaviour of 25 km resistant E. coli, K_{12} transformants on different Km concentrations.

colony No.	0	10	20	30	40	50	60	70	80	90	100	110	120	130	140	150	160	170	180	190	200
1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-
2	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-
5	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
9	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-
10	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-
14	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
15	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
16	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-
17	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
18	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
19	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-
20	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-
21	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
22	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-
23	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-
24	+	+	+	+	+	+	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-
25	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-

Km : kanamycin

Table (15) The growth behaviour of 25 Sm resistant E. coli K_{12} transformants on different Sm concentrations.

colony No.	0	10	20	30	40	50	60	70	80	90	100	110	120	130	140	150	160	170	180	190	200
1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-
2	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-
5	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-
6	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-
8	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
9	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-
10	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
12	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-
13	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
14	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-
15	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-
16	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-
17	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
18	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
19	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-
20	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
21	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-
22	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
23	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
24	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-
25	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-

Sm = streptomycin

3- Gentamicin :-

Table(16) presents the growth behavior of 25 Gm. resistant *E. coli*, k12 transformants on different Gm concentrations. It shows great variability in the level of resistance. Two transformants resist only 20 µg Gm/ml medium,2 transformants resist 30 µg Gm/ml medium, one transformants resist 40 µg Gm/ml medium,two transformants resist 50 µg Gm/ml medium, one transformants resist 60 µg Gm/ml medium, two transformants resist 70 µg Gm/ml medium, two transformants resist 80 µg Gm/ml medium,three transformants resist 90 µg Gm/ml medium, one transformants resist 110 µg Gm/ml medium, two transformants resist 120 µg Gm/ml medium, one transformants resist 140 µg Gm/ml medium, two transformants resist 150 µg Gm/ml medium, two transformants resist 180 µg Gm/ml medium and two transformants resist 200 µg Gm/ml medium. These results might indicate that either each plasmid can give a resistant level of 10 µg Gm/ml medium or some gene interactions are existed giving 20 µg resistance level from one plasmid or 110 µg level from 10 plasmids and so on. The first possibility is more probable, i.e each plasmid add 10 µg Gm level of resistance.

Fig. (4) diagram showing *E.coli*, K12 transformants resistance level to gentamicin.

Table (16) The growth behaviour of 25 Gn resistant E. coli, K_{12} transformants on different Gn concentrations.

colony No.	0	10	20	30	40	50	60	70	80	90	100	110	120	130	140	150	160	170	180	190	200
1	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-
3	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-
6	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
7	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-
8	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-
9	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-
10	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-
11	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
12	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
13	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-
14	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
15	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
16	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
17	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
18	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
19	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
21	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-
22	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-
23	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-
24	+	+	+	-	-	-	-	-	-	+	+	+	+	+	+	-	-	-	-	-	-
25	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Gn = gentamicin

4- Tobramycin :-

Table (17) presents the growth behavior of 25 Tm resistant *E.coli*, K12 transformants on different Tm. concentrations. It shows great variability in the level of resistance. Nine transformants resist only 10μg Tm/ml medium, three transformants resist 20 μg Tm/ml three transformants resist 30μg Tm/ml medium, one transformant resist 40 and another resist 50 μg Tm/ml medium, one transformant resists 70 μg Tm/ml, one transformant resists 80 μg Tm/ml, one transformant resists 90 μg Tm/ml, one transformant resists 110 μg Tm/ml, one transformant resists 130 μg Tm/ml, one transformant resists 140 μg Tm/ml, one transformant resists 150 μg Tm/ml and one transformant resist 180 μg Tm/ml medium. These results also indicate that each plasmid add a level of 10 μg Tm/ml medium resistance. The possibility of gene interactions or regulatory genes can also be existed.

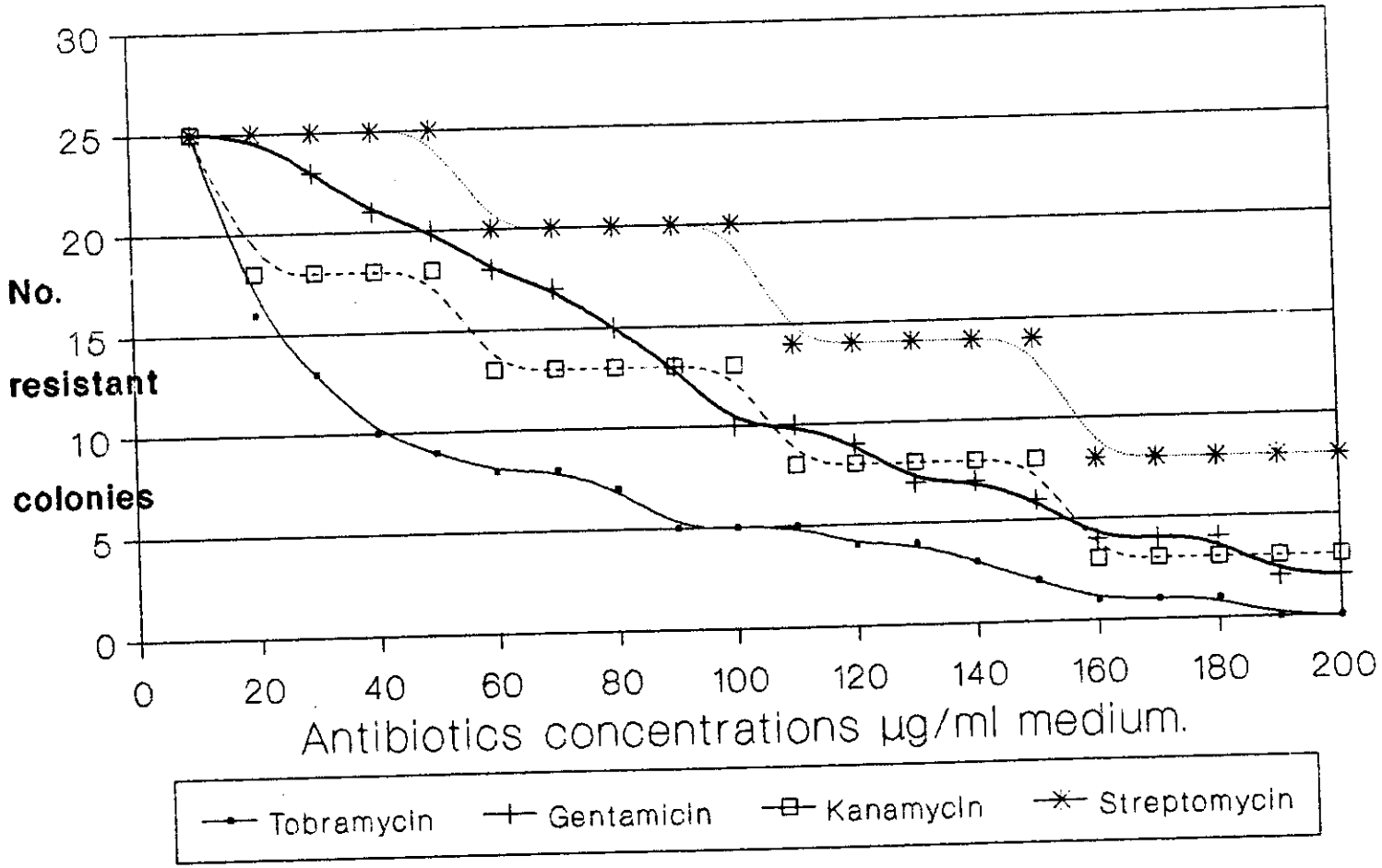
Fig. (4) diagram showing *E.coli*, K12 transformants resistance level to tobramycin.

Table (17) The growth behaviour of 25 Tm resistant E. coli, K_{12} transformants on different Tm concentrations.

colony No.	0	10	20	30	40	50	60	70	80	90	100	110	120	130	140	150	160	170	180	190	200
1	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-
3	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-
4	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
7	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-
9	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
15	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
16	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-
17	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
18	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
19	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
21	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
22	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
23	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-
24	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
25	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Tm : tobramycin

Fig (4) diagram showing single colonies resistance levels to different antibiotics.



The Relationship between Isolate Phage Content and Various Characters:-

Table (18) presents Ps.aeruginosa isolate no, bilharziasis, cancer bacterial counts, No. of spontaneous plaques and following lysis induction with UV & acridine hydrochloride and resistance to antibiotics; 10μg Tm, 30 μg km & 10 μg Sm & 10 μg Gm estimated as zone of inhibition /mm, MIC for each antibiotic and resistance mode of inheritance for each antibiotic.

Table (18) shows that bilharziasis, cancer, high bacterial counts and high phage contents are correlated. However, no clear correlation can be noticed between phage contents and the resistance of any of the antibiotics studied.

Table (18) : P. aeruginosa isolates No., bilharziasis, cancer, bacterial counts, number of spontaneous Plaques & following lysis induction with UV & acridine hydrochloride and resistance to antibiotics; 10 µg Tn, 30 µg Km, 10 µg Sm & 10 µg Gm estimated as zone of inhibition /mm, MIC for each antibiotic & resistance mode of inheritance for each antibiotic.

Isolate No	Bil ♯	Cancer	Bact. count ♯ 105	No. of Plaques			Resistance to antibiotics											
				spont.	UV	acridine	Sm			Km			Gm			Tn		
							I	II	III	I	II	III	I	II	III	I	II	III
1	-	-	1.4	8	120	24	3	>400	p	5	>400	p	6	400	p	4	400	p
2	-	-	1.2	16	160	40	13	50	s	17	100	s	10	100	chron	15	50	s
3	+	+	2.7	240	600	840	10	>400	p	8	400	p	12	50	s	13	100	s
4	+	+	7	22	680	160	4	>400	p	6	>400	p	8	50	p	14	100	s
5	+	+	2.3	280	800	140	6	200	p	10	100	p	10	100	p	12	25	s

I : zone of inhibition/mm II : MIC /µg/ml III : mode of inheritance

S : sensitive P : plasmid chron : chromosomal Bil ♯ : Bilharziasis

Plasmid Curing :-

A- The Effect of Elevated Temperature:-

Cultures of isolate I, resistant to; Sm, Km, Gm and Tm, were grown in liquid nutrient broth at; 42, 45 and 50°C overnight with shaking. Samples of 0.1 ml with suitable dilutions were plated on nutrient agar plates. The plates were incubated at 37°C.Single colonies were tested for their resistance to different antibiotic concentrations.

Complete lethality was obtained by incubation at 50°C. Table (19) presents the resistance levels of 25 single colonies obtained following incubation at 42°C on the different antibiotics concentrations.

Table (19) showed great reduction in resistance levels to the different antibiotics concentrations following incubation at 42°C especially for the two antibiotics Sm and Km. This indicates curing by raising the temperature degree.

Table(20) illustrates the resistance levels of 25 single colonies from isolate no.I obtained following incubation at 45°C on the different antibiotics concentrations. It showed greater reductions in resistance levels to the different antibiotics concentrations following incubation at 45°C than obtained after incubation at 42°C. This indicates greater reduction in

plasmids, i.e plasmid curing by increasing the degree of temperature during incubation.

B- The Effect of Ascorbic acid :-

Cultures of isolate I, resistant to, Sm, Km, Gm and Tm, were grown in liquid nutrient broth supplemented with, 100, 200, 300, 400 and 500 µg ascorbic acid/ml medium overnight with shaking. Samples of 0.1 ml with suitable dilutions from each ascorbic acid concentration were plated on nutrient agar plates. The plates were incubated at 37°C and 25 colonies from each ascorbic acid concentration were tested for their resistance to different antibiotic concentrations.

Tables 21, 22, 23, 24, and 25 showed great reduction in resistance to the various antibiotics. These reduction increased by increasing ascorbic acid concentrations indicating plasmid curing which increase parallel by increasing ascorbic acid concentrations.

C- The Effect of Acridine hydrochloride:

Cell suspensions of isolate I resistant to, Sm, Km, Gm and Tm in saline (0.85 % NaCl) were treated with different acridine hydrochloride concentrations, 0.05, 0.1 and 0.2 mg/ml cell suspension for half an hour at 37°C. Samples of 0.1 ml with suitable dilutions were plated on nutrient agar. The plates were incubated at

37°C for 24 hours. Twenty five colonies from each concentration were tested on different antibiotic concentrations.

Tables 26, 27 and 28 presents the resistant levels of 25 single colonies on different antibiotics concentrations. They showed great reduction in the resistant levels for all antibiotics. This reduction increased by increasing the concentration of acridine hydrochloride. This might indicate the plasmid reduction by increasing acridine hydrochloride concentration.

Table (19): The resistance level of 25 single colonies on the four antibiotics; Sm, Km, Gm and Tm, after growing cultures overnigth at 42 °C

Colony No.	Control 0	Sm conc. µg/ml				Km conc. µg/ml				Gm conc. µg/ml				Tm conc. µg/ml			
		25	50	100	200	25	50	100	200	25	50	100	200	25	50	100	200
1	+	-	-	-	-	+	-	-	-	+	+	+	-	+	+	+	-
2	+	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-
3	+	+	-	-	-	+	-	-	-	+	+	-	-	+	+	-	-
4	+	+	+	-	-	+	+	-	-	+	+	+	-	+	+	+	-
5	+	+	-	-	-	+	-	-	-	+	+	-	-	+	+	-	-
6	+	-	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-
7	+	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-
8	+	+	-	-	-	+	-	-	-	+	+	+	+	+	+	+	-
9	+	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-
10	+	-	-	-	-	-	-	-	-	+	+	-	-	+	+	-	-
11	+	+	-	-	-	+	-	-	-	+	+	+	+	+	+	+	-
12	+	+	+	+	-	+	+	+	-	+	+	+	-	+	+	+	-
13	+	+	+	-	-	+	+	-	-	+	-	-	-	+	-	-	-
14	+	-	-	-	-	-	-	-	-	+	+	-	-	+	+	-	-
15	+	+	-	-	-	+	-	-	-	+	+	-	-	+	+	-	-
16	+	+	-	-	-	+	-	-	-	+	+	+	-	+	+	+	-
17	+	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+
18	+	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-
19	+	+	-	-	-	+	-	-	-	+	+	+	-	+	+	+	-
20	+	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+
21	+	+	-	-	-	+	-	-	-	+	+	-	-	+	+	-	-
22	+	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-
23	+	+	-	-	-	+	-	-	-	+	-	-	-	+	-	-	-
24	+	+	+	+	-	+	+	+	-	+	+	+	-	+	+	+	+
25	+	-	-	-	-	-	-	-	-	+	+	-	-	+	-	-	-
♯	+	18	9	2		19	9	2		25	22	10	4	25	21	10	3

♯ = No. of Resistant colonies

Km = kanamycin Gm = gentamicin Sm = streptomycin Tm = tobramycin

Table (20): The resistance levels of 25 single colonies from isolate no. 1 on the four antibiotics; Sm, Km, Gm and Tm, after growing cultures overnigth at 45°C

Colony No.	Control 0	Sm conc. µg/ml				Km conc. µg/ml				Gm conc. µg/ml				Tm conc. µg/ml			
		25	50	100	200	25	50	100	200	25	50	100	200	25	50	100	200
1	+	-	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-
2	+	+	-	-	-	+	-	-	-	+	-	-	-	+	-	-	-
3	+	-	-	-	-	-	-	-	-	+	+	-	-	+	+	-	-
4	+	-	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-
5	+	+	+	-	-	+	+	-	-	+	-	-	-	+	-	-	-
6	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	+	-	-	-	-	-	-	-	-	+	+	-	-	+	+	-	-
8	+	+	-	-	-	+	-	-	-	+	-	-	-	+	-	-	-
9	+	-	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-
10	+	-	-	-	-	-	-	-	-	+	+	-	-	+	+	-	-
11	+		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	+	-	-	-	-	-	-	-	-	+	+	-	-	+	+	-	-
13	+	-	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-
14	+	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
15	+	-	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-
16	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
17	+	-	-	-	-	-	-	-	-	+	+	-	-	+	+	-	-
18	+	-	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-
19	+	+	-	-	-	+	-	-	-	+	+	+	-	+	+	+	-
20	+	-	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-
21	+	-	-	-	-	-	-	-	-	+	+	-	-	+	+	-	-
22	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
23	+	-	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-
24	+	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
25	+	-	-	-	-	-	-	-	-	+	+	-	-	+	+	-	-
♯	+	6	1			6	1			19	8	1		19	8	1	

♯ : No. of Resistant colonies

Km : kanamycin Gm : gentamicin Sm : streptomycin Tm : tobramycin

Table (21): The resistance levels of 25 single colonies on the four antibiotics; Sm, Km, Gm and Tm, after growing cultures at conc. of 100 µg/ml. of ascorbic acid

Colony No.	Control 0	Sm conc. µg/ml				Km conc. µg/ml				Gm conc. µg/ml				Tm conc. µg/ml			
		25	50	100	200	25	50	100	200	25	50	100	200	25	50	100	200
1	+	-	-	-	-	-	-	-	-	+	+	+	-	+	+	+	-
2	+	+	+	+	+	+	+	+	-	+	+	-	-	+	+	-	-
3	+	+	+	+	-	+	+	+	-	+	+	+	-	+	+	+	+
4	+	+	+	+	-	+	+	+	-	+	+	+	-	+	+	+	-
5	+	+	+	-	-	-	-	-	-	+	+	+	-	+	+	+	-
6	+	-	-	-	-	+	+	+	-	+	+	+	-	+	+	-	-
7	+	+	+	+	-	+	+	+	-	+	+	-	-	+	+	-	-
8	+	+	+	-	-	+	+	-	-	+	+	+	-	+	+	+	-
9	+	+	+	-	-	+	+	-	-	+	-	-	-	+	+	-	-
10	+	+	+	+	-	+	+	+	-	+	+	-	-	+	+	+	+
11	+	+	+	+	+	-	-	-	-	+	+	+	-	+	+	-	-
12	+	-	-	-	-	+	+	+	+	+	+	-	-	+	+	-	-
13	+	+	+	-	-	+	+	+	-	+	+	+	-	+	+	+	-
14	+	+	+	-	-	-	-	-	-	+	+	+	+	+	+	+	+
15	+	+	+	+	-	+	+	+	-	+	+	-	-	+	+	-	-
16	+	+	+	-	-	+	+	-	-	+	+	+	-	+	+	+	+
17	+	-	-	-	-	+	+	-	-	+	+	-	-	+	+	+	-
18	+	+	+	-	-	-	-	-	-	+	+	-	-	+	+	-	-
19	+	+	+	+	-	+	+	-	-	+	+	+	-	+	+	+	-
20	+	+	+	-	-	+	+	+	-	+	+	-	-	+	+	-	-
21	+	+	+	+	-	-	-	-	-	+	+	+	-	+	+	+	-
22	+	+	+	-	-	+	+	+	-	+	-	-	-	+	-	-	-
23	+	-	-	-	-	+	+	-	-	+	+	-	-	+	-	-	-
24	+	+	+	+	-	+	+	-	-	+	+	+	-	+	+	+	-
25	+	+	+	-	-	-	-	-	-	+	+	+	+	+	+	+	+
♯	+	20	20	10	3	18	18	11	1	25	23	14	3	25	23	14	5

♯ : No. of Resistant colonies

Km : kanamycin Gm : gentamicin Sm : streptomycin Tm : tobramycin

Table (22): The resistance levels of 25 single colonies on the four antibiotics after growing cultures at conc. of 200 µg/ml. of ascorbic acid

Colony No.	Control 0	Sm conc. µg/ml				Km conc. µg/ml				Gm conc. µg/ml				Tm conc. µg/ml			
		25	50	100	200	25	50	100	200	25	50	100	200	25	50	100	200
1	+	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-
2	+	+	+	-	-	+	+	-	-	+	-	-	-	-	-	-	-
3	+	-	-	-	-	-	-	-	-	+	+	-	-	+	+	+	-
4	+	+	-	-	-	+	+	-	-	-	-	-	-	+	+	-	-
5	+	+	+	-	-	+	-	-	-	+	+	+	-	+	-	-	-
6	+	-	-	-	-	+	+	-	-	+	+	-	-	+	+	-	-
7	+	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-
8	+	+	-	-	-	+	-	-	-	-	-	-	-	+	+	+	-
9	+	+	+	-	-	-	-	-	-	+	+	+	+	+	-	-	-
10	+	-	-	-	-	-	-	-	-	+	+	+	-	+	+	-	-
11	+	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-
12	+	+	+	-	-	+	+	-	-	+	+	+	-	+	+	-	-
13	+	-	-	-	-	-	-	-	-	+	+	-	-	+	+	+	-
14	+	+	-	-	-	-	-	-	-	+	+	-	-	+	-	-	-
15	+	-	-	-	-	+	-	-	-	-	-	-	-	+	+	-	-
16	+	+	+	-	-	-	-	-	-	+	+	+	-	+	+	-	-
17	+	+	+	-	-	+	+	+	-	+	+	-	-	+	+	+	-
18	+	-	-	-	-	-	-	-	-	+	+	+	-	+	+	-	-
19	+	+	-	-	-	+	-	-	-	+	+	+	+	+	+	+	+
20	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
21	+	-	-	-	-	-	-	-	-	+	+	-	-	+	+	-	-
22	+	+	+	-	-	+	+	-	-	+	+	+	-	+	+	-	-
23	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
24	+	+	+	-	-	-	-	-	-	+	+	-	-	+	+	-	-
25	+	-	-	-	-	-	-	-	-	+	+	-	-	+	+	-	-
♦	+	13	8			10	6	1		19	18	9	2	21	17	5	1

♦ = No. of Resistant colonies

Km = kanamycin Gm = gentamicin Sm = streptomycin Tm = tobramycin

Table (23): The resistance levels of 25 single colonies on the four antibiotics after growing cultures at conc. of 300 µg/ml. of ascorbic acid

Colony No.	Control 0	Sm conc. µg/ml 25	50	100	200	Km conc. µg/ml 25	50	100	200	Gm conc. µg/ml 25	50	100	200	Tm conc. µg/ml 25	50	100	200
1	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	+	+	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-
3	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
4	+	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-
5	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
6	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
7	+	+	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-
8	+	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-
9	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	+	-	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-
11	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
12	+	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-
13	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14	+	+	-	-	-	-	-	-	-	+	+	+	-	+	+	-	-
15	+	-	-	-	-	+	-	-	-	-	-	-	-	+	+	-	-
16	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
17	+	-	-	-	-	+	-	-	-	+	+	-	-	+	-	-	-
18	+	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-
19	+	-	-	-	-	-	-	-	-	+	+	+	-	+	-	-	-
20	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
21	+	-	-	-	-	-	-	-	-	+	+	-	-	+	+	-	-
22	+	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-
23	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
24	+	+	-	-	-	-	-	-	-	+	+	-	-	+	+	-	-
25	+	-	-	-	-	-	-	-	-	+	+	-	-	+	+	-	-
♦	+	5				5				9	8	2		15	9		

♦ = No. of Resistant colonies

Km = kanamycin Gm = gentamicin Sm = streptomycin Tm = tobramycin

Table (24): The resistance levels of 25 single colonies on the four antibiotics after growing cultures at conc. of 400 µg/ml. of ascorbic acid

Colony No.	Control 0	Sm conc. µg/ml				Km conc. µg/ml				Gm conc. µg/ml				Tm conc. µg/ml			
		25	50	100	200	25	50	100	200	25	50	100	200	25	50	100	200
1	+	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-
2	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
4	+	-	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-
5	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
10	+	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-
11	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
12	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
16	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
17	+	+	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-
18	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
19	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20	+	+	-	-	-	+	-	-	-	+	-	-	-	-	-	-	-
21	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
22	+	+	+	-	-	+	+	-	-	-	-	-	-	+	+	-	-
23	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
24	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
25	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
*	+	5	1			4	1			3				5	4		

* : No. of Resistant colonies

Km : kanamycin Gm : gentamicin Sm : streptomycin Tm : tobramycin

Table (25): The resistance levels of 25 single colonies on the four antibiotics after growing cultures at conc. of 500 µg/ml. of ascorbic acid

Colony	Control	Sm conc. µg/ml				Km conc. µg/ml				Gm conc. µg/ml				Tm conc. µg/ml			
No.	0	25	50	100	200	25	50	100	200	25	50	100	200	25	50	100	200
1	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
3	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	+	-	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-
6	+	-	-	-	-	-	-	-	-	-	-	-	-		-	-	-
7	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
8	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
14	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
16	+	-	-	-	-	-	-	-	-	-	-	-	-		-	-	-
17	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
18	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
19	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
21	+	+	-	-	-	-	-	-	-	-	-		-	-	-	-	-
22	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-		-
23	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-		-
24	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
25	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
*	+	3				3				4				2	4		

* : No. of Resistant colonies

Km : kanamycin Gm : gentamicin Sm : streptomycin Tm : tobramycin

Table (26): The resistance levels of 25 single colonies on the four antibiotics after growing cultures at conc. of 0.05 mg/ml. acridine hydrochloride

Colony No.	Control 0	Sm conc. µg/ml				Km conc. µg/ml				Gm conc. µg/ml				Tm conc. µg/ml			
		25	50	100	200	25	50	100	200	25	50	100	200	25	50	100	200
1	+	-	-	-	-	-	-	-	-	+	+	-	-	+	+	-	-
2	+	+	+	+	-	+	+	+	-	+	+	+	-	+	+	-	-
3	+	+	+	-	-	+	+	-	-	+	+	+	-	+	+	-	-
4	+	+	+	-	-	+	+	-	-	+	+	-	-	+	-	-	-
5	+	+	+	+	-	+	+	+	-	+	+	+	+	+	+	+	-
6	+	+	+	-	-	+	+	-	-	+	+	-	-	+	-	-	-
7	+	+	+	+	-	+	+	+	-	+	+	+	-	+	+	-	-
8	+	+	+	-	-	+	+	-	-	+	+	+	-	+	+	-	-
9	+	+	+	-	-	-	-	-	-	+	-	-	-	+	-	-	-
10	+	+	-	-	-	+	-	-	-	+	+	-	-	+	+	-	-
11	+	-	-	-	-	+	-	-	-	+	+	+	-	+	+	-	-
12	+	+	+	+	-	+	+	+	-	+	+	-	-	+	+	-	-
13	+	+	+	-	-	+	-	-	-	+	+	+	-	+	+	-	-
14	+	+	+	-	-	+	+	-	-	+	+	+	+	+	+	+	-
15	+	+	-	-	-	+	-	-	-	+	+	-	-	+	+	-	-
16	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	-
17	+	+	+	-	-	+	+	-	-	+	-	-	-	+	-	-	-
18	+	+	+	+	-	+	+	+	-	+	+	-	-	+	+	-	-
19	+	+	+	-	-	+	+	+	-	+	+	+	-	+	-	-	-
20	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	-	-
21	+	+	+	-	-	+	+	-	-	+	-	-	-	+	-	-	-
22	+	+	+	+	-	+	+	+	-	+	+	-	-	+	+	-	-
23	+	+	+	-	-	+	+	-	-	+	+	+	-	+	-	-	-
24	+	+	+	+	+	+	+	+	+	+	-	-	-	+	+	-	-
25	+	+	+	-	-	+	+	-	-	+	+	-	-	+	-	-	-
‡	+	23	21	9	3	23	19	10	3	25	21	11	2	25	17	3	

‡ : No. of Resistant colonies

Km : kanamycin Gm : gentamicin Sm : streptomycin Tm : tobramycin

Table (27): The resistance levels of 25 single colonies on the four antibiotics the after effect of 0.1 ng/ml.acridine hydrochloride

Colony No.	Control 0	Sm conc.μg/ml 25	50	100	200	Km conc.μg/ml 25	50	100	200	Gm conc.μg/ml 25	50	100	200	Tm conc.μg/ml 25	50	100	200
1	+	-	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-
2	+	+	+	+	-	+	+	+	-	+	+	+	-	+	+	-	-
3	+	-	-	-	-	-	-	-	-	+	+	-	-	+	+	-	-
4	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
5	+	+	-	-	-	+	-	-	-	+	+	-	-	+	-	-	-
6	+	+	+	+	-	+	+	+	-	+	+	-	-	+	-	-	-
7	+	+	+	-	-	+	+	-	-	+	+	+	-	+	+	-	-
8	+	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
9	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	+	+	+	-	-	+	+	-	-	+	-	-	-	+	-	-	-
11	+	+	+	+	-	+	+	:	-	+	+	-	-	+	+	-	-
12	+	+	+	-	-	+	+	-	-	+	+	+	-	+	+	-	-
13	+	+	+	-	-	+	+	-	-	+	-	-	-	-	-	-	-
14	+	-	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-
15	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
16	+	+	+	-	-	+	+	-	-	+	+	-	-	+	-	-	-
17	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	-
18	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
19	+	+	+	+	-	+	+	+	-	+	+	-	-	+	-	-	-
20	+	-	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-
21	+	+	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-
22	+	+	-	-	-	+	-	-	-	+	-	-	-	-	-	-	-
23	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	-
24	+	+	+	-	-	+	+	-	-	+	+	-	-	+	-	-	-
25	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
♯	+	16	13	6	2	16	13	6	2	21	12	5		16	7	1	

♯ : No. of Resistant colonies

Km : kanamycin Gm : gentamicin Sm : streptomycin Tm : tobramycin

Table (28): The resistance levels of 25 single colonies on the four antibiotics the after effect of 0.2 mg/ml. acridine hydrochloride

Colony No.	Control 0	Sm conc. µg/ml				Km conc. µg/ml				Gm conc. µg/ml				Tm conc. µg/ml			
		25	50	100	200	25	50	100	200	25	50	100	200	25	50	100	200
1	+	+	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-
2	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
3	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	+	+	+	-	-	+	+	-	-	+	-	-	-	-	-	-	-
6	+	+	-	-	-	+	+	-	-	+	-	-	-	+	-	-	-
7	+	-	-	-	-	-	-	-	-	+	-	-	-	+	+	-	-
8	+	+	-	-	-	+	-	-	-	+	-	-	-	-	-	-	-
9	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	+	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-
11	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
12	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
13	+	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
14	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
16	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
17	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
18	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
19	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20	+	+	-	-	-	+	-	-	-	+	-	-	-	-	-	-	-
21	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
22	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
23	+	-	-	-	-	+	-	-	-	+	-	-	-	-	-	-	-
24	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
25	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
‡	+	6	1			9	3			8				4	1		

‡ : No. of Resistant colonies

Km : kanamycin Gm : gentamicin Sm : streptomycin Tm : tobramycin

DISCUSSION