R E S U L T S

In the present investigation, 50 strains of Ps. aeruginosa were isolated from 235 patients examined in three different sources.

Table (3) summerize place of isolation and clinical sources of obtained strains.

All isolates were gram negative, actively motile bacilli. On solid medium they revealed:-

- All grow readily at 42°C and optimaly at 37°C. Different colonies especially large and small ones, with dark greyish center and irregular translucent edges,

- They produced colored pigments after incubation at 37°C for 24 hr.

- They were oxidase positive rapidly producing purple color with 0.5% W/V N, N- dimethyl - P- phenylene diamine monohydrochloride.

- Slime and surface pellicle were formed when grown in nutrient broth and gelatin was liquefied.

Table (3) shows also, that, high frequency of isolation of Ps. aeruginosa was observed among patients of General Hospital of Zifta (as 25 strains were isolated from 100 patients) however, the incidence of such organism among Benha University Hospital patients was relatively low (as 25 strains were obtained from 135 patients).

Five strains were isolated from burn cases (3 from Zifta and 2 from Benha), 13 strains from septic wound cases (7 from Zifta and 6 from Benha) and 32 strains from patients suffering from urinary tract infections (15 from Zifta and 17 from Benha).

Table (3): Sources and places of isolation of 50 strains of *Ps. aeruginosa*.

Strain No.	Source	Strain No.	Source
1	S.W.	26	S.W.
2	Burn	27	U.T.I.
3	U.T.I.	28	S.W.
4	U.T.I.	29	U.T.I.
5	Burn	30	S.W.
6	U.T.I.	31	U.T.I.
7	S.W.	32	U.T.I.
8	S.W.	33	U.T.I.
9	U.T.I.	34	U.T.I.
10	Burn	35	Burn
11	U.T.I.	36	S.W.
12	S.W.	37	U.T.I.
13	U.T.I.	38	U.T.I.
14	U.T.I.	39	U.T.I.
15	S.W.	40	U.T.I.
16	U.T.I.	41	U.T.I.
17	S.W.	42	Burn.
18	S.W.	43	U.T.I.
19	U.T.I.	44	U.T.I.
20	U.T.I.	45	S.W.
21	U.T.I.	46	U.T.I.
22	U.T.I.	47	U.T.I.
23	U.T.I.	48	S.W.
24	U.T.I.	49	U.T.I.
25	U.T.I.	50	U.T.I.

* Strains from 1 to 25 from Zifta.
* Strains from 26 to 50 from Benha.
* U.T.I. = Urinary tract infection.
* S.W. = Septic wound.

The resistance patterns of the isolated strains of Ps. aeruginosa is presented in table (4). As indicated from the table, 37 strains (74%) were shown to be colimycin - resistant, 25 strains (50%) were gentamicin-resistant, 21 strains (42%) were cefotaxime - resistant, 19 strains (38%) were polymyxin - B - resistant, 13 strains (26%) were carbenicillin - resistant and 6 strains (12%) were tobramycin resistant. Amikacin was the most effective antibiotic as 90% of the tested strains were sensitive to it.

All strains except 6 strains were resistant to one or more antibiotic e.g. 9 strains (18%) resisted one antibiotic, 11 strains (22%) resisted two antibiotic, 10 strains (20%) resisted three antibiotics and 14 strains (28%) were resistant to more than 3 antibiotic.

Table (4): Antibiotic resistance patterns of the isolated strains of Ps. aeruginosa:

Strain No.	Antibiotic resistance marker	Strain No.	Antibiotic resistance marker
1	GM	26	PB - CL - CTX
2	CL	27	PB - CTX - CL
3	GM - CTX	28	GM - CTX - CL
4	-	29	NN,PB,GM,CL,CB
5	GM - CL	30	NN, PB, GM, CL
6	PB-GM-CTX-CL-CB	31	CL - PB
7	GM - CTX	32	CL
8	AN-NN-GM-CTX-CL	33	PB - CL
9	GM - CL	34	PB - CL - CB
10	CTX - CL	35	CTX - CL
11	CL	36	PB - CL
12	AN - GM - CTX - CL	37	GM, CTX - CL
13	PB - GM - CTX - CB	38	GM, CTX, CL - CB
14	AN,NN,PB,GM,CTX,CL,CB	39	CL
15	AN, NN, PB, GM - CL	40	PB - GM - CL - CB
16	AN, GM, CB	41	PB-GM-CTX-CL-CB
17	CL	42	GM - CTX - CL
18	NN, GM, CL	43	PB - CTX - CL
19	GM - CTX - CB	44	PB - CL
20	PB - CL	45	CTX
21	-	46	-
22	PB - GM - CL - CB	47	-
23	PB - GM - CTX - CL-CB	48	-
24	CL	49	CL
25	GM - CTX - CL - CB	50	-

GM: Gentamicin - CL: Colimycin - CTX: Cefotaxime
PB: Polymyxin B - AN: Amikacin - CB : Carbenicillin and
NN: Tobramycin
- = Sensitive

By using the method of Gillies and Govan (1969) it was possible to type 46 strains (92%) out of the 50 strains tested. The most predominant pyocin types could be arranged in the following descending order type 31(6 strains = 12%), type N.T (4 strains = 8%) and types 17 and 49 (3 strains for each = 6%). Eight strains (16%) showed inhibition patterns which could not be classified according Govan's inhibition patterns shown in table (2), they were designated unclassified types (U.C). Twenty six pyotypes could be recognized among the tested 50 isolates. The Discriminatory ability was 6, as six pyocin types represented 50% of the isolates (Table 5).

Table (5): Pyocin typing of the isolated strains of Ps. aeruginosa by using Gillies and Govan's method.

Strain No.	Pyotype	Strain No.	Pyotype
1	17	26	17
2	25	27	U.C
3	68	28	73
4	31	29	48
5	25	30	N.T
6	51	31	9
7	31	32	U.C
8	17	33	22
9	49	34	10
10	31	35	49
11	95	36	79
12	31	37	N.T
13	9	38	U.C
14	U.C.	39	33
15	31	40	5
16	N.T	41	U.C
17	N.T	42	49
18	U.C	43	12
19	86	44	2
20	13	45	63
21	61	46	87
22	31	47	2
23	61	48	103
24	73	49	3
25	U.C	50	U.C

U.C = Unclassified
N.T = Non typable

Table (6) correletes pyocin typing by Gillies and Govan's method and antibiotic resistance. It could be concluded that all strains of pyocin types 49 (3 strains), 25 (2 strains) and 73 (2 strains) were colimycin resistant, however the majority of the pyocin types U.C - N.T - 31 and 17 were colimycin resistant as 87.5%, 75%, 67% - 67% of its strains respectively were resistant to colimycin. Regarding gentamicin resistance (25 strains = 50%), the most predominent pyocin types were U.C (5 strains), 31 (4 strains) and N.T (3 strains). Concerning cefotaxime resistant strains, the pyotypes U.C - 31 and 49 were the most predominating ones, as 5,3 and 2 strains were shown respectively. No correlation between pyocin types and tobramycin resistance as there was no predominating pyotypes. On the other hand, the six sensitive strains showed no predominant pyocin types.

Table (6): Correlation between antibiotic resistance pattern and pyocin typing using Gillies and Govan's method.

Antibiotic	Strain No.	Pyotype	Antibiotic	Strain No.	Pyotype	Antibiotic	Strain No.	Pyotype	Antibiotic	Strain No.	Pyotype
Amikacin ✱ (5)	8	17	Colimycin ✱ (37)	2	25	Tobramycin ✱ (6)	8	17	Carbenicillin ✱ (13)	6	N.T
	12	31		5	25		14	U.C		13	62
	14	U.C		6	51		15	31		14	58
	15	31		8	17		18	U.C		16	42
	16	N.T		9	49		29	48		19	67
Cefotaxime ✱ (21)	3	68		10	31		30	N.T		22	73
	6	51		11	95	Gentamicin ✱ (25)	1	17		23	12
	7	31		12	31		3	68		25	U.C
	8	17		14	U.C		5	25		29	65
	10	31		15	31		6	51		34	U.C
	12	31		17	N.T		7	31		38	43
	13	9		18	U.C		8	17		40	47
	14	U.C		20	13		9	49		41	19
	19	86		22	31		12	31	Polymyxin ✱ (19)	6	51
	23	61		23	61		13	9		13	9
	25	U.C		24	73		14	U.C		14	U.C
	26	17		25	U.C		15	31		15	31
	27	U.C		26	17		16	N.T		20	13
	28	73		27	U.C		18	U.C		22	31
	35	49		28	73		19	86		23	61
	37	N.T		29	48		22	31		26	17
	38	U.C		30	N.T		23	61		27	U.C
	41	U.C		31	9		25	U.C		29	48
	42	49		32	U.C		28	73		30	N.T
	43	12		33	22		29	48		31	9
	45	63		34	49		30	N.T		33	22
				35	49		37	N.T		34	49
				36	79		38	U.C		36	79
				37	N.T		40	5		40	5
				38	U.C		41	U.C		41	U.C
				39	33		42	49		43	12
				40	5					44	2
				41	U.C						
				42	49						
				43	12						
				44	2						
				49	3						

Table (7) Correletes both geographic and clinical sources with pyocin typing using Gillies and Govan method. The pyocin types 31 (6 strains) and 25 (2 strains) were only restricted to Zifta strains however no pyocin type could be detected in Benha strains only. On the other hand it was difficult to correlate the clinical source of a strain with it's pyotype however the two strains isolated from burns at Benha University Hospital were of the pyotype 49 this reflects cross infection in between patients of Benha. Two strains out of 3 isolated from burn at General Hospital of Zifta were of the type 25. Sometimes a particular pyotype could be detected at different geographic and various clinical sources such as the pyotype 31 which could be detected in bath urinary and septic wound strains and the pyotype 17 which was detected among both localities Benha and Zifta.

Table (7): Correlation between geographic and clinical sources and pyocin typing of the isolated strains of Ps. aeruginosa using Gillies and Govan's method.

Location	Clinical sources							
	Urinary tract infections				Burn		Septic wounds	
	Strain No.	Pyotype	Strain No.	Pyotype	Strain No.	Pyotype	Strain No.	Pyotype
General Hospital of Zifta	3	68	19	86	2	25	1	17
	4	31	20	13			7	31
	6	51	21	61	5	25	8	17
	9	49	22	31			12	31
	11	95	23	61	10	31	15	31
	13	9	24	73			17	N.T
	14	U.C	25	U.C			18	U.C
	16	N.T						
University Hospital of Benha	27	U.C	44	2			26	17
	29	48	46	87	35	49	28	73
	31	9	47	2			30	N.T
	32	U.C	49	3	42	49	36	79
	33	22	50	U.C			45	63
	34	10					48	103
	37	N.T						
	38	U.C						
	39	33						
	40	5						
	41	U.C						
	43	12						

Table (8). Correletes the most predominant pyotypes by Gillies and Govan technique and antibiotic resistance pattern. There was no solid correlation between both parameters, however amikacin and tobramycin resistances were detected among the types U.C - 31 - N.T and 17 but not in the type 49. Five strains out of 8 strains of the type U.C showed gentamicin resistance.

Table (8): Correlation between the most predominant pyocin types by Gillies and Govan's method and antibiotic resistance patterns.

Pyocin type	Strain No.	Antibiotic resistance
U.C (8)	14	AN - NN - PB-GM - CTX - CL - CB
	18	NN - GM - CL
	25	GM - CTX - CL - CB
	27	PB - CTX - CL
	32	CL
	38	GM - CTX - CL - CB
	41	PB - GM - CTX - CL - CB
	50	-
31 (6)	4	-
	7	GM - CTX
	10	CTX - CL
	12	A N - GM - CTX - CL
	15	AN - NN - PB - GM - CL
	22	PB - GM - CL - CB
N.T (4)	16	AN - GM - CB
	17	CL
	30	NN - PB - GM - CL
	37	GM - CTX - CL
17 (3)	1	GM
	8	AN - NN - GM - CTX - CL
	26	PB - CTX - CL
49 (3)	9	GM - CL
	35	CTX - CL
	42	GM - CTX - CL

- = Sensitive

Table (9) summerizes the different properties of the selected constituting the proposed indicator set for pyocin typing. Five strains were isolated from urinary tract infections, two strains from septic wounds and a strain from burn. They showed different antibiotic resistance patterns and produced different pyocins when typed by the method of Gillies and Govan 1969.

Table (9): Properties of the selected Ps. aeruginosa strains for the proposed pyocin typing set.

Strain No.	Code No.	Clinical source	Geographic source	Antibiotic resistance pattern	Pyocin type by Gillies & Govan method
1	I	Septic wound	Zifta	GM	17
2	II	Burn	Zifta	CL	25
3	III	urinary tract infection	Zifta	GM, CTX	68
14	IV	urinary tract infection	Zifta	AN,NN,PB,GM,CTX,CL,CB	U.C.
16	V	urinary tract infection	Zifta	AN, GM, CB	N.T.
21	VI	urinary tract infection	Zifta	Sensitive	61
23	VII	urinary tract infection	Zifta	PB, GM, CTX, CL, CB	61
45	VIII	Septic wound	Benha	CTX	63

Tables (10 - 15) show the inhibition patterns of twenty strains when typed by both indicator sets once weekly. Reproducibility of Govan strains ranged from 85 - 100% with a mean value of 95.3% while that of the selected strains ranged from 90 - 97.5% with a mean value of 95.8%.

Statistical analysis using (T) test showed that there was an insignificant difference between the reproducibility of both indicator sets as the calculated (t) was less than the tabulated one at 5% level of significance.

Table (10): Inhibition patterns of 20 strains of Ps. aeruginosa using both Govan indicator strains and the selected ones in the first week.

Strain No.	Govan indicator strains								Strain No.	Selected strains							
	1	2	3	4	5	6	7	8		I	II	III	IV	V	VI	VII	VIII
1	-	-	+	-	-	-	+	-	1	-	-	+	+	+	+	+	+
2	+	-	+	-	-	-	+	-	2	+	-	+	+	+	-	+	+
3	-	-	-	+	-	-	-	-	3	-	+	-	-	-	-	+	-
4	-	-	-	-	-	-	+	-	4	-	+	-	-	-	+	-	-
5	+	-	+	-	-	-	+	-	5	-	-	+	-	+	+	-	+
6	+	+	+	+	-	-	-	+	6	-	-	-	-	-	-	-	-
7	-	-	-	-	-	-	+	-	7	-	-	-	-	-	+	+	-
8	-	-	+	-	-	-	+	-	8	+	-	+	+	+	-	+	+
9	-	-	+	-	+	-	-	-	9	+	+	+	+	-	-	+	+
10	-	-	-	-	-	-	+	-	10	+	+	+	+	+	-	+	+
11	+	-	+	-	-	+	+	-	11	+	+	+	+	+	+	+	+
12	-	-	-	-	-	-	+	-	12	-	-	+	+	+	-	+	+
13	-	-	-	-	+	-	+	-	13	-	+	+	-	-	+	+	-
14	+	-	-	+	-	-	+	-	14	-	+	+	+	+	-	+	+
15	-	-	-	-	-	-	+	-	15	-	-	+	-	-	-	-	+
16	-	-	-	-	-	-	-	-	16	-	-	+	-	-	-	+	+
17	-	-	-	-	-	-	-	-	17	-	-	-	-	-	-	-	-
18	+	+	-	-	-	+	-	-	18	-	-	-	+	+	-	-	+
19	-	+	+	-	-	+	-	-	19	-	-	-	-	-	+	+	-
20	-	-	-	+	-	-	-	+	20	-	-	+	+	-	-	-	+

+ = Inhibition

- = No inhibition

Table (11): Inhibition patterns of 20 strains of Ps. aeruginosa using both Govan indicator strains and the selected ones in the second week.

Strain No.	Govan indicator strains								Selected strains							
	1	2	3	4	5	6	7	8	I	II	III	IV	V	VI	VII	VIII
1	-	-	-	-	-	-	+	-	-	-	+	+	+	+	-	+
2	-	-	+	-	-	-	+	-	-	-	+	+	+	-	-	+
3	-	-	-	+	-	-	-	-	-	+	-	-	-	-	+	-
4	-	-	-	-	-	-	-	-	-	+	-	-	-	+	-	-
5	+	-	-	-	-	-	+	-	-	-	+	-	+	-	-	-
6	-	-	-	+	-	-	-	+	-	-	-	-	-	-	-	-
7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
8	-	-	+	-	-	-	+	-	+	-	+	+	+	-	+	+
9	-	-	+	-	+	-	-	-	+	+	+	+	-	-	+	+
10	-	-	-	-	-	-	+	-	+	+	-	+	-	-	+	+
11	+	-	-	-	-	+	+	-	-	-	+	+	+	+	+	+
12	-	-	-	-	-	-	+	-	-	-	+	+	+	-	+	-
13	-	-	-	-	+	-	-	-	-	+	+	-	-	-	+	-
14	-	-	-	+	-	-	+	-	-	+	-	-	-	-	+	+
15	-	-	-	-	-	-	+	-	-	-	+	-	-	-	-	+
16	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+	+
17	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
18	-	+	-	-	-	-	+	-	-	-	-	+	-	-	-	+
19	-	+	-	-	-	-	+	-	-	-	-	-	-	-	+	-
20	-	-	-	+	-	-	-	-	-	-	+	+	-	-	-	+

\+ = Inhibition

\- = No inhibition

Table (12): Inhibition patterns of 20 strains of Ps. aeruginosa using both Govan indicator strains and the selected ones in the third week.

Strain No.	Govan indicator strains								Selected indicator strains							
	1	2	3	4	5	6	7	8	I	II	III	IV	V	VI	VII	VIII
1	-	-	+	-	-	-	+	-	-	-	+	+	+	+	+	+
2	+	-	+	-	-	-	+	-	+	-	+	+	+	-	+	+
3	-	-	-	+	-	-	-	-	-	+	-	-	-	-	+	-
4	-	-	-	-	-	-	+	-	-	+	-	-	-	+	-	-
5	+	-	+	-	-	-	+	-	-	-	+	-	+	+	-	+
6	+	+	+	+	-	-	-	+	-	-	-	-	-	-	-	-
7	-	-	-	-	-	-	+	-	-	-	-	-	-	+	+	-
8	-	-	+	-	-	-	+	-	+	-	+	+	+	-	+	+
9	-	-	+	-	+	-	-	-	+	+	+	+	-	-	+	+
10	-	-	-	-	-	-	+	-	+	+	+	+	+	-	+	+
11	+	-	+	-	-	+	+	-	+	+	+	+	+	+	+	+
12	-	-	-	-	-	-	+	-	-	-	+	+	+	-	+	+
13	-	-	-	-	+	-	+	-	-	+	+	-	-	+	+	-
14	+	-	-	+	-	-	+	-	-	+	+	+	+	-	+	+
15	-	-	-	-	-	-	+	-	-	-	+	-	-	-	-	+
16	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+	+
17	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
18	+	+	-	-	-	+	-	-	-	-	-	+	+	-	-	+
19	-	+	+	-	-	+	-	-	-	-	-	-	-	+	+	-
20	-	-	-	+	-	-	-	+	-	-	+	+	-	-	-	+

\+ = Inhibition

\- = No inhibition

Table (13): Inhibition patterns of 20 strains of *Ps. aeruginosa* using both Govan indicator strains and the selected ones in the fourth week.

Strain No.	Govan indicator strains								Selected indicator strains							
	1	2	3	4	5	6	7	8	I	II	III	IV	V	VI	VII	VIII
1	-	-	-	-	-	-	+	-	-	-	+	+	+	+	+	+
2	-	-	+	-	-	-	+	-	+	-	+	+	+	-	+	+
3	-	-	-	+	-	-	-	-	-	+	-	-	-	-	+	-
4	-	-	-	-	-	-	-	-	-	+	-	-	-	+	-	-
5	+	-	-	-	-	-	+	-	-	-	+	-	+	-	-	+
6	-	+	-	+	-	-	-	+	-	-	-	-	-	-	-	-
7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
8	-	-	+	-	-	-	+	-	+	-	+	+	+	-	+	+
9	-	-	+	-	+	-	-	-	+	+	+	+	-	-	+	+
10	-	-	-	-	-	-	+	-	+	+	-	+	-	-	+	+
11	+	-	-	-	-	+	+	-	-	-	+	+	+	+	+	+
12	-	-	-	-	-	-	+	-	-	-	+	+	+	-	+	-
13	-	-	-	-	+	-	+	-	-	+	+	-	-	+	+	-
14	+	-	-	+	-	-	+	-	-	+	+	-	+	-	+	+
15	-	-	-	-	-	-	+	-	-	-	+	-	-	-	-	+
16	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+	+
17	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
18	+	+	-	-	-	+	-	-	-	-	-	+	+	-	-	+
19	-	+	-	-	-	+	-	-	-	-	-	-	-	-	+	-
20	-	-	-	+	-	-	-	+	-	-	+	+	-	-	-	+

\+ = Inhibition

\- = No inhibition

Table (14): Inhibition patterns of 20 strains of Ps. aeruginosa using both Govan indicator strains and the selected ones in the fifth week.

Strain No.	Govan indicator strains								Selected indicator strains							
	1	2	3	4	5	6	7	8	I	II	III	IV	V	VI	VII	VIII
1	-	-	+	-	-	-	+	-	-	-	+	+	+	+	+	+
2	+	-	+	-	-	-	+	-	+	-	+	+	+	-	+	+
3	-	-	-	+	-	-	-	-	-	+	-	-	-	-	+	-
4	-	-	-	-	-	-	+	-	-	+	-	-	-	+	-	-
5	+	-	+	-	-	-	+	-	-	-	+	-	+	+	-	+
6	+	+	+	+	-	-	-	+	-	-	-	-	-	-	-	-
7	-	-	-	-	-	-	+	-	-	-	-	-	-	+	+	-
8	-	-	+	-	-	-	+	-	+	-	+	+	+	-	+	+
9	-	-	+	-	+	-	-	-	+	+	+	+	-	-	+	+
10	-	-	-	-	-	-	+	-	+	+	+	+	+	-	+	+
11	+	-	+	-	-	+	+	-	+	+	+	+	+	+	+	+
12	-	-	-	-	-	-	+	-	-	-	+	+	+	-	+	+
13	-	-	-	-	+	-	+	-	-	+	+	-	-	+	+	-
14	+	-	-	+	-	-	+	-	-	+	+	+	+	-	+	+
15	-	-	-	-	-	-	+	-	-	-	+	-	-	-	-	+
16	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+	+
17	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
18	+	+	-	-	-	+	-	-	-	-	-	+	+	-	-	+
19	-	+	+	-	-	+	-	-	-	-	-	-	-	+	+	-
20	-	-	-	+	-	-	-	+	-	-	+	+	-	-	-	+

\+ = Inhibition

\- = No inhibition

Table (15): Reproducibity (% R) of indicator strains of Ps. aeruginosa.

% R	Govan indicator strains								Selected indicator strains							
	1	2	3	4	5	6	7	8	I	II	III	IV	V	VI	VII	VIII
2nd. week	80	90	75	100	100	90	95	95	90	95	90	95	85	80	90	90
3rd. week	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
4th. week	90	100	75	100	100	100	90	100	95	95	95	95	95	85	100	95
5th. week	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Mean	85	97.5	87.5	100	100	97.5	96.3	98.8	96.3	97.5	96.3	97.5	95	90	97.5	96.5

Table (16) shows that the selected indicator set was able to classify 45 strains (90%) out of the tested 50 strains of Ps. aeruginosa. There were 28 pyocin types, the most predominant pyocin types could be arranged in the following descending order; type Uc´ (5 strains = 10%) - N·T´ (5 strains = 10%), 65´ (4 strains = 8% - 67´ & 73´ (3 strains for each = 6%). The discriminatory ability was equal to 8 i,e 8 pyocin types represent 50% of the tested strains.

Table (16): Pyocin typing of the isolated strains of *Ps. aeruginosa* by using selected indicator strains.

Strain No.	Pyocin type	Strain No.	Pyocin type
1	79	26	86
2	18	27	19
3	73	28	N.T.
4	73	29	65
5	U.C.	30	9
6	N.T.	31	43
7	67	32	69
8	18	33	20
9	105	34	U.C.
10	10	35	65
11	10	36	42
12	24	37	U.C.
13	69	38	49
14	58	39	87
15	64	40	47
16	42	41	19
17	N.T.	42	24
18	32	43	36
19	67	44	53
20	65	45	67
21	64	46	U.C.
22	73	47	65
23	12	48	89
24	103	49	N.T.
25	U.C.	50	N.T.

Table (17) correlates antibiotic resistance and pyocin typing for the tested strains of Ps. aeruginosa. No solid correlation could be detected, however the most predominating pyotypes N.T´ and U.C´ were detected among strains resistant to colimycin as 4 out of 5 strains were detected and gentamicin resistant strains as 3 out of the 5 (U.C´) strains and 2 out of 5 (N.T´) strains could be observed. Resistance to other tested antibiotics showed no significant relationship to pyocin types by this method.

Table (17): Correlation between antibiotic resistance and pyocin typing of the tested Ps. aeruginosa strains by using selected indicator strains.

Antibiotic	Strain No.	pyotype
Amikacin * (5)	8	18
	12	24
	14	64
	15	58
	16	42
Cefotaxime * (21)	3	69
	6	N.T
	7	87
	8	18
	10	10
	12	24
	13	62
	14	58
	19	67
	23	76
	25	U.C
	26	86
	27	19
	28	N.T
	35	65
	37	U.C
	38	43
	41	19
	42	24
	43	36
	45	67

Antibiotic	Strain No.	Pyotype
Colimycin * (37)	2	10
	5	U.C
	6	N.T
	8	18
	9	105
	10	10
	11	10
	12	24
	14	58
	15	64
	17	N.T
	18	32
	20	65
	22	73
	23	12
	24	103
	25	U.C
	26	86
	27	19
	28	N.T
	29	65
	30	9
	31	43
	32	69
	33	20
	34	U.C
	35	65
	36	42
	37	U.C
	38	43
	39	87
	40	47
	41	19
	42	24
	43	36
	44	2
	49	3

Antibiotic	Strain No.	Pyotype
Tobramycin * (6)	8	18
	14	58
	15	64
	18	32
	29	65
	30	9
Gentamicin * (25)	1	79
	3	69
	5	U.C
	6	N.T
	7	87
	8	18
	9	105
	12	24
	13	62
	14	58
	15	64
	16	42
	18	32
	19	67
	22	73
	23	12
	25	U.C
	28	N.T
	29	65
	30	9
	37	U.C
	38	43
	40	47
	41	19
	42	24

Antibiotic	Strain No.	Pyotype
Carbenicillin * (13)	6	N.T
	13	62
	14	58
	16	42
	19	67
	22	73
	23	12
	25	U.C
	29	65
	34	U.C
	38	43
	40	47
	41	19
Polymyxin	6	N.T
	13	62
	14	58
	15	64
	20	65
	22	73
	23	12
	26	86
	27	19
	29	65
	30	9
	31	43
	33	20
	34	U.C
	36	42
	40	47
	41	19
	43	36
	44	53

* Number of resistant strains.

Table (18) shows the correlation between both clinical and geographic sources and pyocin typing by the selected indicator strain set. It was clear that the strains belonging to the type 7́3 were isolated from patients with urinary tract infection located at Zifta Hospital. On the other hand all strains belonging to pyocin types 19́ and 43́ (2 strains for each were isolated from strains of urinary tract infections isolated from Benha University Hospital. The pyotype N.T́ and U.Ć were detected among strains obtained from both localities and different clinical sources.

Table (18): Correlation between geographic and clinical sources and pyocin typing of the isolated strains of Ps. aeruginosa using the selected indicator strains.

Location	Clinical sources							
	Urinary tract infections				Burn		Septic wounds	
	Strain No.	Pyotype	Strain No.	Pyotype	Strain No.	Pyotype	Strain No.	Pyotype
Zifta	3	73´	19	67´	2	10´	1	79´
	4	73´	20	65´	3	U.C´	7	87´
	6	N.T´	21	64´	10	10´	8	18´
	9	105´	22	73´			12	24´
	11	10´	23	12´			15	64´
	13	62´	24	103´			17	N.T.´
	14	58´	25	U.C			18	32´
	16	42´						
Benha	27	19´	44	53´			26	86´
	29	65´	46	U.C´	35	65´	28	N.T.´
	31	43´	47	65´	42	24´	30	9´
	32	69´	49	N.T´			36	42´
	33	20´	50	N.T´			45	67´
	34	U.C´					48	89´
	37	U.C´						
	38	43´						
	39	87´						
	40	47´						
	41	19´						
	43	36´						

Table (19) shows that the antibiotic resistance patterns of the most frequent pyocin types using the proposed typing set. The strains belonging to the pyotype 67́ showed resistance to one or more antibiotic, however, sensitive strains to all tested antibiotics were observed among the types N.t́, U.ć, 65́ and 73́ but not 67́ whose strains showed cefotaxime resistance. No solid relationship could be detected between antibiotic resistance pattern and pyocin typing using the proposed method.

Table (19): Correlation between the most predominant pyocin types by selected indicator strains and antibiotic resistance patterns of the isolated Ps. aeruginosa strains.

Pyocin type	Strain No.	Antibiotic resistance pattern
N.T (5)	6	PB - GM - CTX - CL - CB
	17	CL
	28	GM - CTX - CL
	49	CL
	50	-
U.C (5)	5	GM - CL
	25	GM - CTX - CL - CB
	34	PB - CL - CB
	37	CL
	46	-
65 (4)	20	PB - CL
	29	NN - PB - GM - CL - CB
	35	CTX - CL
	47	-
73 (3)	3	GM - CTX
	4	-
	22	PB - GM - CL - CB
67 (3)	7	GM - CTX
	19	GM - CTX - CB
	45	CTX

- Sensitive

DISCUSSION

Ps. aeruginosa is one of the gram negative bacteria which has been isolated from many parts of the human body in addition to animals, soil and water. It is recognised as the etiological agent of a variety of human infections. If there is a suitable typing method for differentiation of Ps. aeruginosa, it is easier to detect the sources of nosocomial infections and to establish proper treatment for the patients (Sakamoto et al., 1975).

Antibiotic resistance of the isolated strains as shown in table (4) revealed the presence of multiresistant strains, 24 out of 50 strains were multiresistant i,e resisted three or more antibiotics. This may be due to the spread of plasmids among the tested strains leading to the dissimination of multiresistant organisms which cause serious infections to man (Falkiner et al., 1982). Moody et al., (1970) found that 90% of the tested 332 strains of Ps. aeruginosa were kanamycin resistant followed by streptomycin (25%) and neomycin (22%). On the

other hand, Cervants - Vega et al., (1986) studied antibiotic resistance of 322 strains of Ps. aeruginosa, they studied streptomycin, gentamicin, tobramycin and carbenicillin, the resistances to these antibiotics were arranged in the following descending order 74, 13, 8 and 7% respectively. No amikacin resistance was found. The most common resistance patterns were streptomycin, streptomycin gentamicin and tobramycin, gentamicin streptomycin. Their finding differ slightly from results shown in table (4), this may be attributed to the excessive use of antibiotics in Egyptian hospitals (Del-Piano et al., 1986 and Allen et al., 1987).

Epidemiological typing of strains of Ps. aeruginosa can be done by a variety of techniques, including biotyping (Bobo et al., 1973 and Pitt 1980), serologic typing (Young and Moody 1974 & Brokopp et al., 1977), bacteriophage typing (Farmer & Herman 1969 - Edmonds et al., 1972 and Bergen, 1973), pyocin typing (Gillies and Govan 1966- Bergen 1968 - Zabransky & Dray 1969, Jones et al., 1974,

Govan 1978 and Brokopp & Farmer 1979), and antimicrobial susceptibility patterns (Bobo et al., 1973 and Brokopp et al., 1977). Of these procedures, antimicrobial susceptibility is most commonly performed, but serotyping is most often used for epidemiologic purposes. Pyocin typing may be used in conjunction with serotyping because it generally yields a higher percentage of typable strains and is more discriminating (Edmonds et al., 1972 and Brokopp et al., 1979). Thus, pyocin typing may differentiate among serologically identical strains. Unfortunately, pyocin typing is less reproducible than serotyping, and this, along with the information of most investigators to rely more on serologic patterns than on pyocin patterns, makes pyocin typing less available.

The two major techniques used for typing by pyocin production are those of Gillies and Govan (Gillies and Govan 1966 & Govan and Gillies 1969) and Jones et al., (1974). In their original form, these two systems shared a common problem in that at least 48 hours was needed to

produce results. However, Fyfe et al., (1984) described a modified procedure which requires less time and can be applied to mucoid strains as well.

If pyocin typing could be made more reproducible, it might be of more value than serologic typing. However, the selection of a set of indicator strains for pyocin typing is always difficult and often made quite subjectively, primarily because there are a variety of factors which must be considered, such as stability of cultures, lack of bacteriophages and ease of reading zones of inhibition. Further, the existence of two major, but different methods and sets of indicator strains has not contributed to standardization of techniques for pyocin typing of Ps. aeruginosa.

The present investigation compare between Gillies and Govan typing set and a selected indicator strains set and attempts to develop a new set which might prove to be more reproducible and discriminating than either set alone.

Three critical factors were analyzed in order to select the new pyocin typing set:

(1) The reproducibility of pyocin patterns generated by indicator strains.

(2) The ability of different sets of indicator strains to discriminate among different strains of Ps. aeruginosa.

(3) The percentage of typable isolates.

The first two factors were expected to affect one another (Schable et al., 1986).

If the field of Ps. aeruginosa pyocin typing, the codification of patterns was initially empirical (Urbano and Boddi, 1977); authors who developed new typing methods assigned a short tag to each of the patterns they recognized and accorded it the dignity of "type". This served their purpose of concision but had serious drawbacks, in that other workers who followed their methods

inevitably came accross new patterns and were faced with the problem of cooding them. Thus, Darrell and Wahba, (1964) used a set of 12 indicator strains and established 11 provisional pyocin types, corresponding with reaction patterns reported in a table and designated by capital letters; strains that gave negative reactions on all 12 indicators were considered untypable. Zabransky and Day (1969) used seven of the Darrell and Wahba indicators, together with four others, and were forced to define eight additional "Mayotypes" and four subtypes.

Shriniwas (1974) using eight of Wahba's original indicators, found 154 inhibition patterns; he retained the original letter code for the patterns following the Darrell and Wahba types, and assigned a numerical code to 20 other frequently encountered patterns.

With such procedure a good deal of information was lost; for instance, it was impossible from the published reports to understand which patterns appart from the most common ones, could be detected in a sample of isolates.

Also, one may not calculate the frequency of positive reactions given by one particular indicator strain, nor assess the discriminatory power of each indicator. Furthermore, identical patterns may give different designation by different authors, making it difficult to compare the result even when the same indicators and typing methods have been used. The codification reported by Govan (1978) was universally applied and gave a high percent of reproducibility, for this reason, it was considered in coding the inhibition patterns obtained when using the selected indicator strain set in typing the isolated strains of Ps. aeruginosa.

Of the 50 cultures available in this study, 8 strains were selected randomly for to develop a set of indicator strains for pyocin typing (table 9). Reproducibility was determined for Gillies and Govan and these selected strains was determined by weekly repeated typing for 20 strains out of the isolated 50 strains six times (table 10 - 14). Statistical analysis using (t) test showed that there is no significant difference in the

results obtained with the two sets of indicator cultures (table 15).

One of the major problems faced when attempting to develop an alternative set of indicator strains was that a selecting strains which were easy to interpret and were reproducible, without sacrificing discrimination. The indicator strains which were difficult to read were more likely to be poorly in reproducibility studies, Schable et al. (1986) found in their strudies that the indicator strains that were the most difficult to interpret and least reproducible were among the most discriminating one. Astable pyocin type patterns is necessary to obtain consistent results over various periods of times. Changes in inhibition patterns can be caused by the indicator strain (Merrikin and Tirry 1972), producer strain (Govan, 1978), growth conditions (Bergen, 1972 - Brokopp and Farmer, 1979), or a combination of these or other factors. Although it was not possible to differentiate absolutely between these factors.

Using both sets in pyocin typing of the isolated strains, it was clear that the percentage of typable strains with both sets of indicator strains (Gillies and Govan) 92% and the selected 90% either was comparable to or exceeded that observed by others (Edmonds et al.,1972, Bruun et al., 1976, Govan 1978 and Schable et al., 1986). The discriminatory potential of the selected indicator set is 33.3% greater than that of the Gillies and Govan set (table 5 and 16). However, the problem of non typability has been encountered by various workers in the range of 3.3 to 31% depending on various factors like source of the strains and methods of typing (Shriniwas, 1976). The typability of the pyocin typing system has been augumented by induction by mitomycin - C (Tripathy and Chadwick, 1971) as well as by the use of additional indicator strains (Govan and Gillies 1969 - Gadde et al., 1980 and Senqupta 1980 and 1982).

The most common pyotypes among the tested strains of

Ps. aeruginosa according to the method of Gillies and Govan (1969) were 31 (12%) followed by type 17 and 49 which yeild for each, these findings (table 5) were in accordance with that found by others (Heckman et al.,1972, Govan 1978, Porbska 1979 - Conroy et al., 1983, and Chitakara and Feierabend 1987) who stated that the most common pyotypes among their isolates of Ps. aeruginosa were 1, 3, 5, 10 and 31. On the other hand the selected indicator set for pyocin typing revealed other pyotypes such as 6$\acute{5}$ (8%) and 6$\acute{7}$ and 7$\acute{3}$ (6%) as the most common types (table 16). This could be explained by strain variations leading to differences in pyocin production among strains isolated in Egypt and those isolated elsewhere.

Regarding the correlation between antibiotic resistance and pyocin types, Cervants - Vega et al., (1987) found that resistance of Ps. aeruginosa to either tobramycin, gentamicin or carbenicillin was found mainly in strains producing the pyocin type 10. The present investigation (table 6 & 8) showed that colimycin resistance

was detected mainly among strains of the types 49, 25 and 73 (by the Gillies and Govan method), however, amikacin and tobramycin resistance were detected among the types U.C. - 31 and 17 but not in the type 49. It was interesting to mention that 5 out of 8 strains produced unclassified patterns (U.C.) were gentamicin - resistant.

The proposed method could not correlate significantly between the antibiotic resistance and pyocin types tables (16 - 17 and 19), however, the most predominating type (U.C) was produced by strains resistant to colimycin (4 out of 5 strains, while gentamicin resistance was detected among 3 out of 5 (U.C.) strains.

Tables (7 and 18) showed the correlation between both geographic and clinical sources of the tested strains of Ps. aeruginosa when typed by both methods. The Gillies and Govan method failed to correlate directly between the above mentioned parameters, however the pyocin types 31 and 25 were restricted only to strains isolated from Zifta General Hospital. On the other hand,

the two strains producing the pyotype 49 were recovered from patients of Benha University Hospital and suffering from burn infections, this may reflect cross infection among patients of burn ward at this hospital. One pyotype could be recovered from different geographic and various clinical sources such as pyotypes 31 and 17.

The proposed method for pyocin typing shows that strains producing the type 73́ were isolated from patients suffering from urinary tract infections located at Zifta General Hospital, however all strains belonging to type 19́ and 43́ were isolated from strains of urinary tract infections isolated from Benha University Hospital (table 18). The role of environment and hospital conditions in transfer of pseudomonal infections (Kelly et al., 1982 and Kapur & Shriniwas 1986).

Combining the two sets typing Ps. aeruginosa resulting in higher typability i,e (98%) as only one strain (No. 17) still non typable, it could be concluded that

the use of additional 8 strains of Ps. aeruginosa improves both reproducibility and discriminatiny ability of the original pyocin typing method of Gillies and Govan.

In a wider context, the question remains as to which is the most suitable typing system for epidemiological studies of Ps. aeruginosa, and realestically, is any one system adequate? Despite the improvement in pyocin typing described in this investigation, the method still does not match the rapidity of the other most suitable typing method, serotyping.

Ps. aeruginosa is serologically heterogenous, and identification of group specific heat stable lipopolysaccharide antigens by agglutination forms the basis of O-serotyping procedures. Several systems have been described and their use reviewed (Kageyama 1975, Lanyi and Bergan 1978 - and Brokopp and Farmer 1979). O-serogroup sera are available commercially, but they are expensive and the most widely used system (Difco) requires a set

of antigen suspensions for characterizing the sera. In addition, the sera can only be purchased as a complete set of 17 sera.

A major disadvantage of O-serogrouping is that the discriminatory power is only fair (Brokopp et al., 1977), further discrimination can be provided by detection of H-antigens, but the procedures for H-typing are beyond the scope of many laboratories (Pitt 1980). The typability of Ps. aeruginosa by O serological typing is usually over 90%, but serotyping is often unsatisfactory for mucoid Ps. aeruginosa in which O-antigens may be masked, typing of colonial dissociants in which serological changes occur within a single culture and typing polyagglutinable Ps. aeruginosa, the latter together with mucoid Ps. aeruginosa forms less than 50% of clinical isolates but is frequently observed in patients with cystic fibrosis (Penketh et al., 1983).

By the use of the improved method of Gillies and Govan in combination with O-serotyping it is concluded