RESULTS

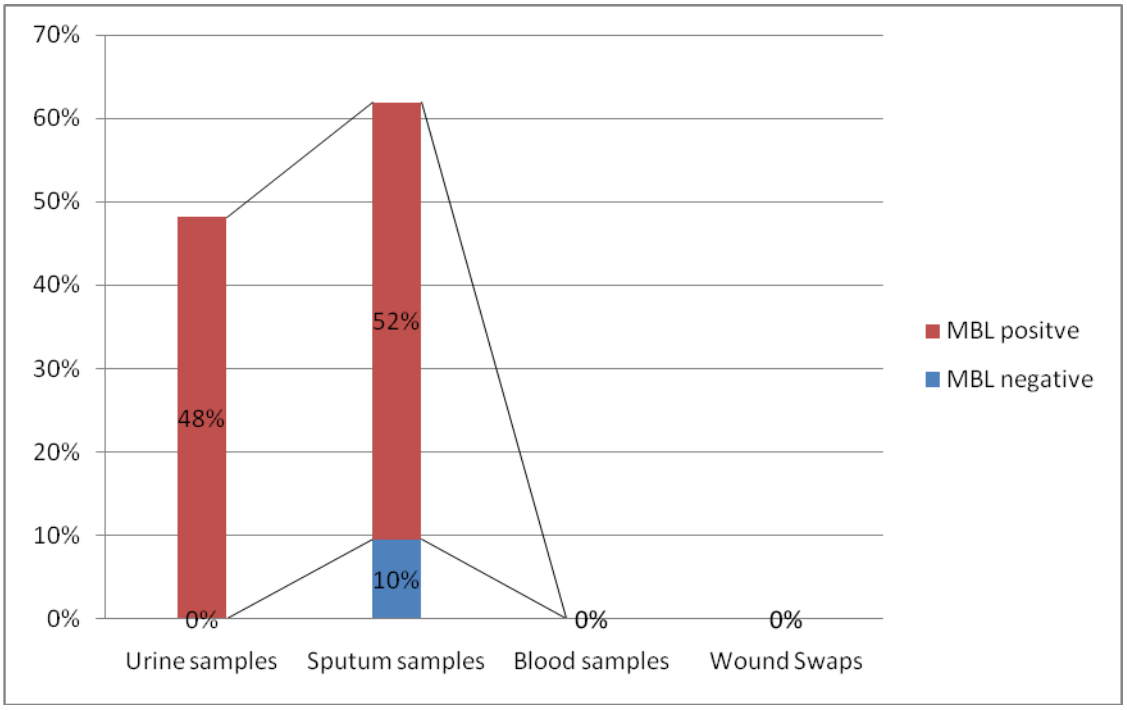
A. SAMPLE RESULTS

Table (8): Isolated *Pseudomonas aeruginosa* strains from patient samples.

Sample type	Positive		Negative	
	N	%	N	%
Sputum samples (n=21)	13	62%	8	38%
Urine samples (n=27)	13	48%	14	52%
Blood samples (n=2)	0	0%	2	100%
Wound swaps (n=2)	0	0%	2	100%
Total (n=52)	26	50%	26	50%

This table shows that 50% of patient samples were positive for *Pseudomonas aeruginosa*. Highest rate of *Pseudomonas* isolation was from sputum samples (62%).

Fig. (6): Frequency of isolation of *Pseudomonas aeruginosa* strains from different types of patient samples



This chart shows that the frequency of isolation of *Pseudomonas aeruginosa* was slightly higher in sputum samples (62%) than in urine samples (48%).

All strains isolated from urine samples and the majority of strains isolated from sputum samples were MBL positive.

Table (9): Isolated *Pseudomonas aeruginosa* strains from Environmental samples

Source	Positive		Negative	
	n	%	*n*	%
Air conditioner (n=4)	2	50%	2	50%
Ambu bag (n=2)	2	100%	0	0%
Antiseptic solutions (n=2)	0	0%	2	100%
Artificial ventilation fluid reservoir (n=10)	2	20%	8	80%
Artificial ventilation machine buttons (n=2)	0	0%	2	100%
Artificial ventilation tubes (n=4)	2	50%	2	50%
Bed (n=2)	0	0%	2	100%
DC shock handles (n=2)	0	0%	2	100%
Floor (n=8)	6	75%	2	25%
Kidney tray (n=6)	2	33%	4	67%
Laryngoscope (n=4)	0	0%	4	100%
Light buttons (n=6)	0	0%	6	100%
Lubrication gel (n=4)	0	0%	4	100%
Patient side drawers (n=4)	2	50%	2	50%
Phone (n=4)	0	0%	4	100%
Sphygmomanometer (n=2)	0	0%	2	100%
Stethoscope (n=2)	2	100%	0	0%
Suction apparatus tubing (n=4)	4	100%	0	0%
Surgical silk (n=2)	0	0%	2	100%

Table (n=2)	0	0%	2	100%
Thermometer (n=2)	0	0%	2	100%
Trolley (n=6)	0	0%	6	100%
Wall oxygen humidifier (n=2)	0	0%	2	100%
Water tap/sink (n=10)	8	80%	2	20%
Total (n=96)	32	33%	64	67%

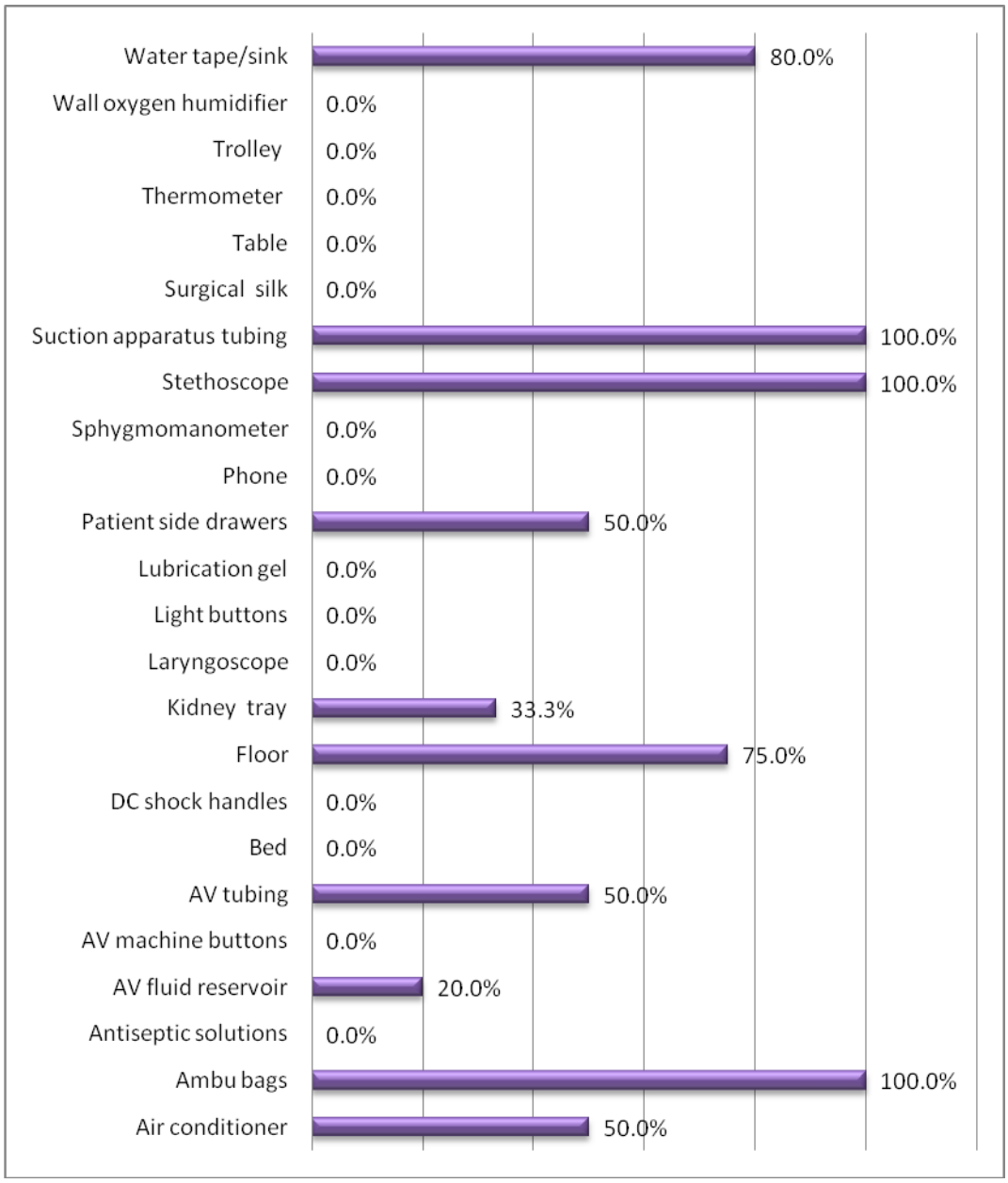
This table shows that 33% of the environmental samples were positive for *Pseudomonas aeruginosa*. The highest frequency of *Pseudomonas* isolation was from Ambu bag (100%), Stethoscope (100%), suction apparatus tubing (100%), water tap/sink (80%) and floor (75%).

Table (10): Staff hand samples that were positive for *Pseudomonas aeruginosa*

	***n* (%)**
Positive	2 (13%)
Negative	14 (87%)
Total	16 (100%)

This table shows that only 13% (n=2) of staff hand samples were positive for *Pseudomonas aeruginosa*.

Fig. (7): Environmental samples that were positive for *Pseudomonas aeruginosa*



This figure shows that highest frequency of *Pseudomonas* isolation from environmental samples was from Ambu bags (100%), Stethoscope (100%), suction apparatus tubing (100%), water tap/sink (80%) and floor (75%).

B. METALLO- B-LACTAMASE AND EXTENDED SPECTRUM B-LACTAMASE PRODUCTION RESULTS

Table (11): Metallo-β-lactamase and extended spectrum β-lactamase production results in isolated *Pseudomonas aeruginosa* strains

	n (%) of positive strains		
	ETest MBL	Meropenem-EDTA double-disk synergy test	ESBL double-disk synergy test
Patient strains (*n* = 26)	24 (92%)	24 (92%)	0 (0%)
Environmental strains (*n* = 32)	6 (19%)	6 (19%)	0 (0%)
Staff hand strains (*n* = 2)	0 (0%)	0 (0%)	0 (0%)
Total (*n* = 60)	30 (50%)	30 (50%)	0 (0%)

Metallo-β-lactamase (MBL) production was tested for by Meropenem-EDTA double-disk synergy test and E-Test MBL, both tests gave equivalent results. MBL production was highest in patient strains (92%), less in environmental strains (19%) and was not detected in staff hand strains.

Extended spectrum β-lactamase (ESBL) production was tested for by double-disk synergy test. ESBLs were not detected in any *Pseudomonas aeruginosa* strain in this study.

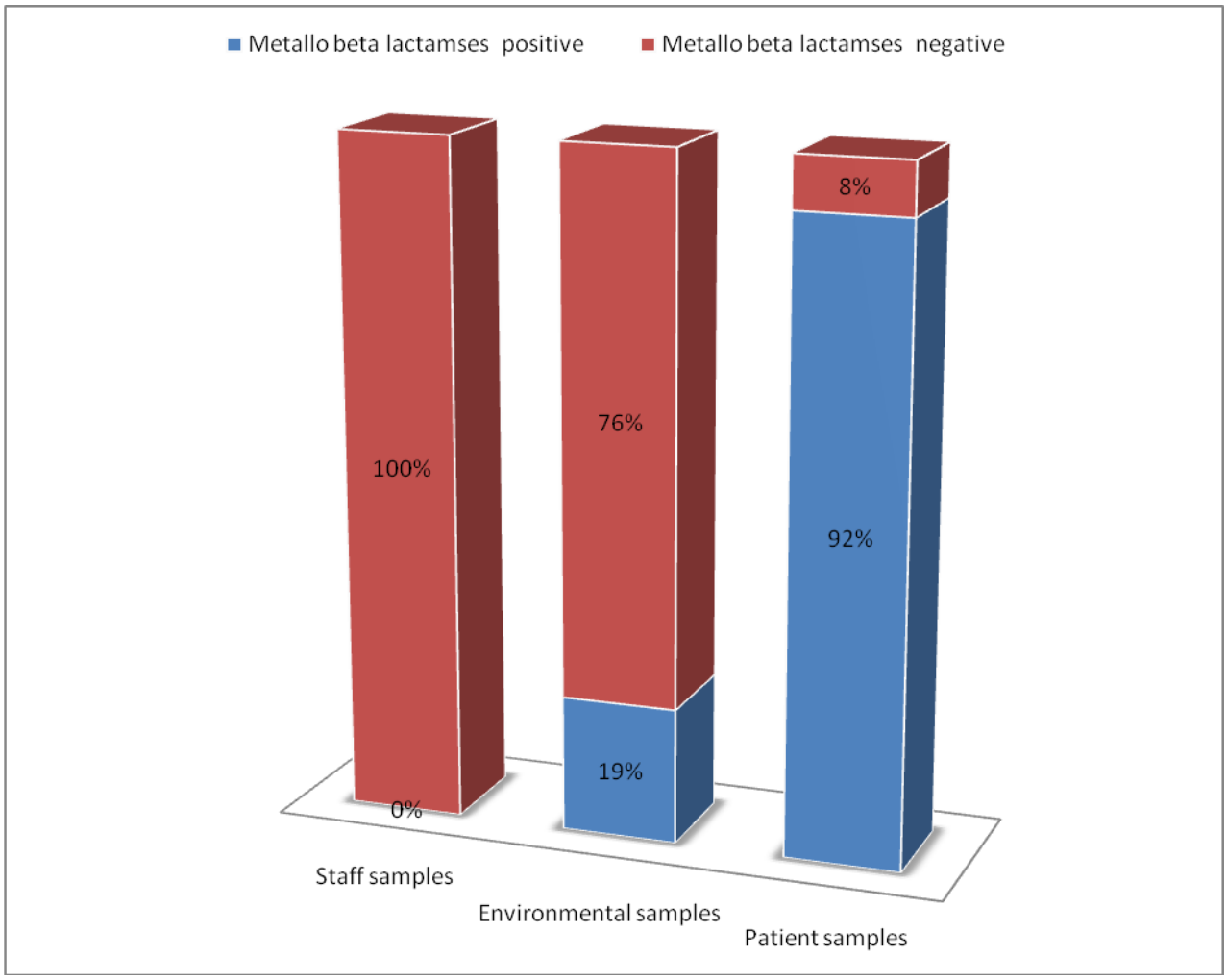
Table (12) Comparison between patient and environment/staff samples for metallo-β-lactamase production

	MBL positive n (%)	MBL negative *n* (%)
Patient samples (*n* = 26)	24 (92%)	2 (8%)
Environmental /staff samples (*n* = 34)	6 (18%)	28 (82%)
Total (*n* = 60)	30 (50%)	30 (50%)

Fisher exact test two sided P value = 1.954 e-7.
P value is < 0.05 which mean that there is a statistically significant difference in MBL distribution between patient and environmental/staff samples.

This table shows that the frequency of MBL production was significantly higher in strains isolated from patient samples when compared to strains isolated from environmental samples.

Fig. (8): Metallo-β-lactamase production rates in patient, environment and staff isolates.



This figure shows striking difference in Metallo-β-lactamase distribution between patient samples in one hand and environmental and staff samples in the other hand. Patient samples showed predominance of metallo-β-lactamase producing strains. On the other hand environmental and staff samples showed predominance of metallo-β-lactamase negative strains.

Table (13): Comparison of antibiotic resistance rates for MBL-producing and non MBL-producing *Pseudomonas aeruginosa* strains

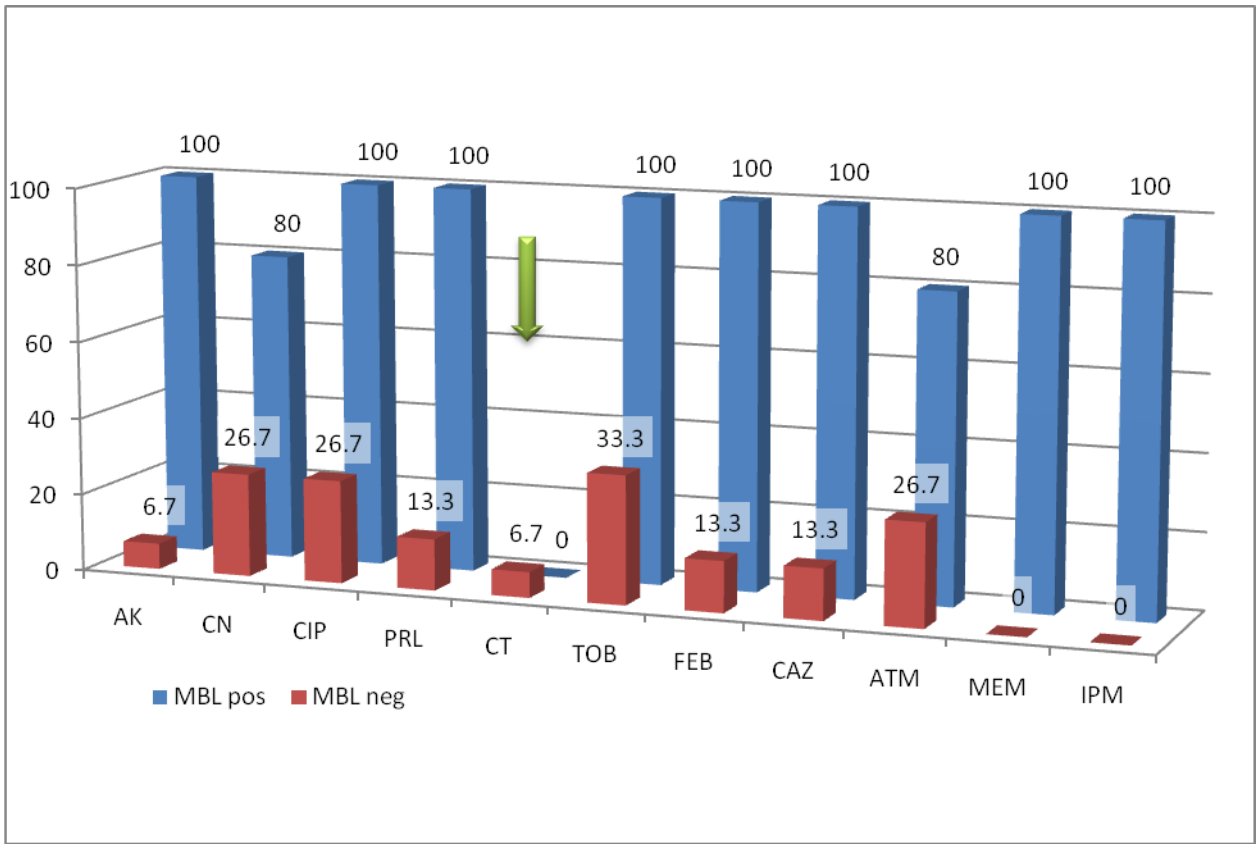
Antibiotic	No. (%) of resistant isolates		
	MBL producing strains (*n* = 30)	Non-MBL producing strains (*n* = 30)	All strains (*n* = 60)
AK	30 (100%)	2 (6.7%)	32 (53.3%)
CN	24 (80%)	8 (26.7%)	32 (53.3%)
CIP	30 (100%)	8 (26.7%)	38 (63.3%)
PRL	30 (100%)	4 (13.3%)	34 (56.7%)
CT	0 (0%)	2 (6.7%)	2 (3.3%)
TOB	30 (100%)	10 (33.3%)	40 (66.7%)
FEB	30 (100%)	4 (13.3%)	34 (56.7%)
CAZ	30 (100%)	4 (13.3%)	34 (56.7%)
ATM	24 (80%)	8 (26.7%)	32 (53.3%)
MEM	30 (100%)	0 (0%)	30 (50%)
IPM	30 (100%)	0 (0%)	30 (50%)

AK, Amikacin; CN, gentamycin; CIP, ciprofloxacin; PRL, piperacillin; CT, Colistin; TOB, Tobramycin; FEB, Cefepime; CAZ, ceftazidime; ATM, aztreonam; MEM, meropenem; IPM, imipenem.

All MBL producing *Pseudomonas* strains were resistant to all tested antibiotics except colistin (0% resistance) and aztreonam (80% resistance rate).

On the other hand non MBL producing strains showed much less resistance, the most frequently effective antibiotics were amikacin and colistin (6.7% resistance rate) followed by piperacillin, cefepime, and ceftazidime (13.3 resistance rate)

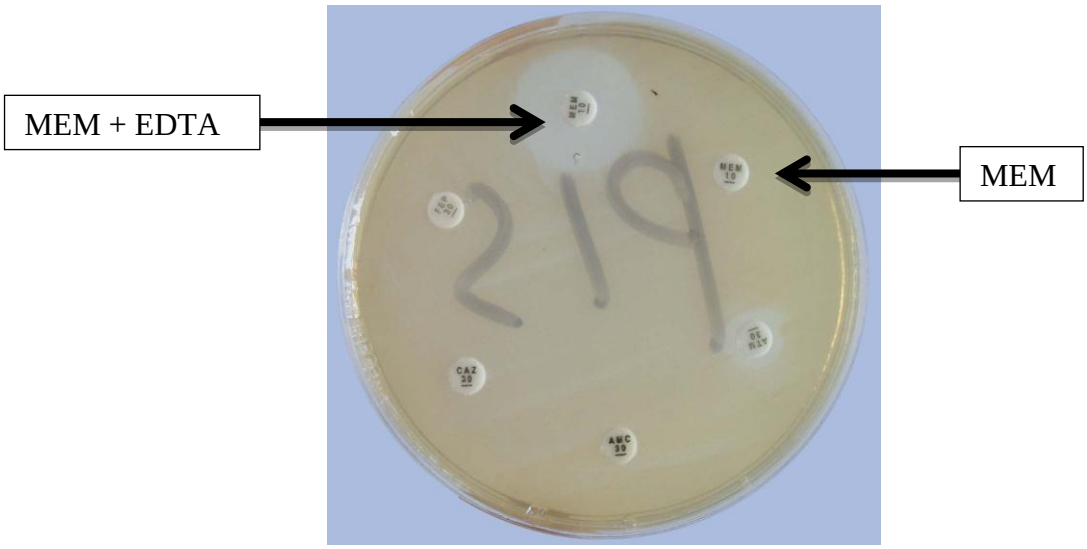
Fig. (9): Comparison of Resistance rates for MBL producing and non MBL producing *Pseudomonas aeruginosa* strains



AK, Amikacin; CN, gentamycin; CIP, ciprofloxacin; PRL, piperacillin; CT, Colistin; TOB, Tobramycin; FEB, Cefepime; CAZ, ceftazidime; ATM, aztreonam; MEM, meropenem; IPM, imipenem.

This chart clearly illustrates the massive difference in antibiotic resistance between MBL producing *Pseudomonas* and non MBL producing *Pseudomonas*. The former is much more resistant to all antibiotics tested. The only exception is CT (colistin) to which nearly all *Pseudomonas* strains (both MBL and non-MBL producing strains) are sensitive. The arrow point to the noticed drop in resistance ratio noticed in CT result.

Fig. (10): A positive Meropenem-EDTA double-disk synergy test



MEM, Meropenem.

This figure shows a positive meropenem EDTA double disc synergy test. A positive test is indicated by increase of ≥7 mm in zone diameter in the presence of EDTA compared to those with MEM tested alone.

Fig. (11): A negative Meropenem-EDTA double-disk synergy test

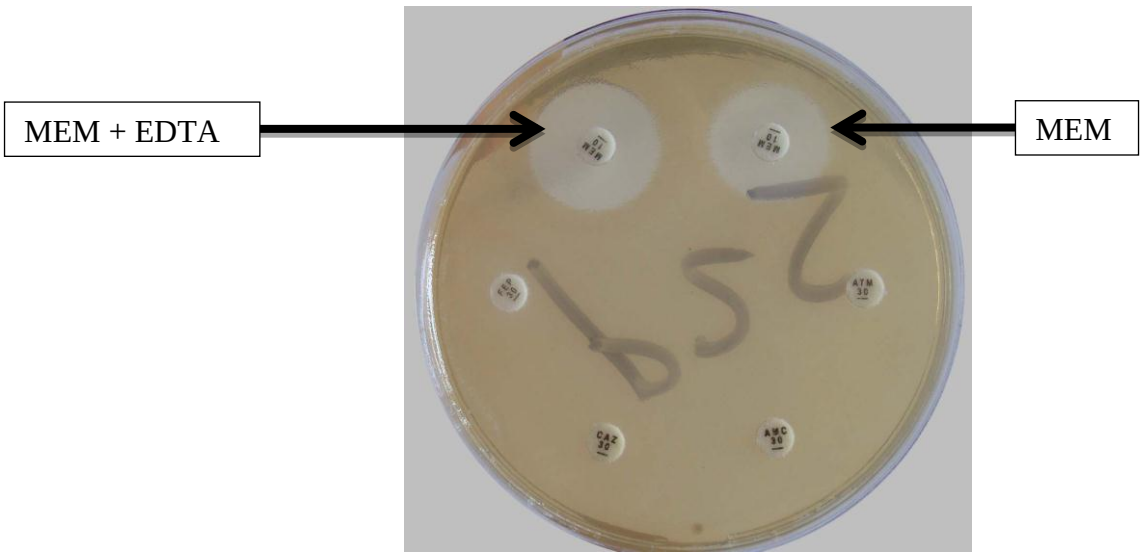
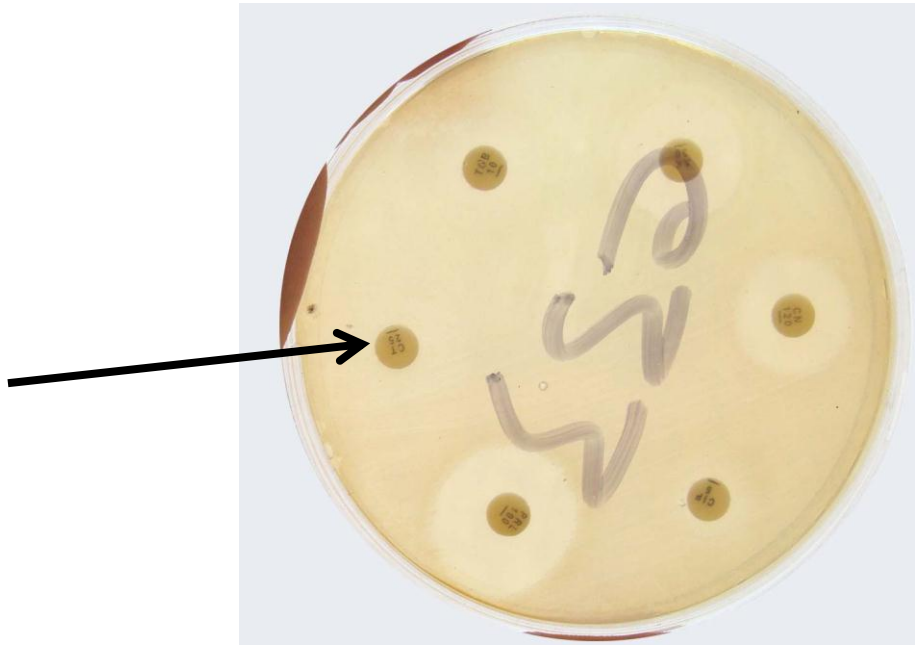
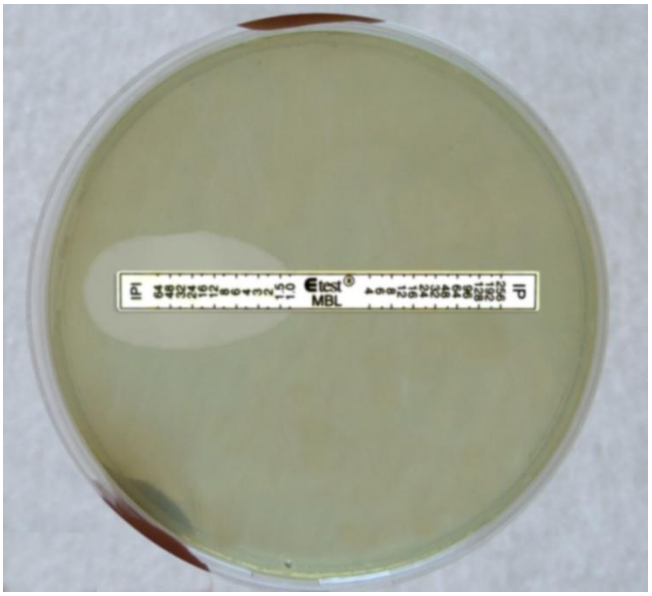


Fig. (12): A colistin resistant Pseudomonas isolate



This strain was found to be colistin resistant, the arrow points to colistin (CT) disc which was immediately surrounded by *Pseudomonas* growth with no zone of inhibition at all.

Fig. (13): A strain positive for MBL Etest



C. ANTIBIOGRAM ANALYSIS

Table (14): Observed patterns of Antibiotic resistance for isolated *Ps. aeruginosa* stains

Pattern	AK	CN	CIP	PRL	CT	TOB	FEB	CAZ	ATM	MEM	IPM
A1	S	S	S	S	S	S	S	S	S	S	S
A2	S	S	**R**	S	**R**	**R**	S	S	S	S	S
A3	**R**	**R**	S	S	S	**R**	S	S	**R**	S	S
A4	S	**R**	**R**	S	S	**R**	S	S	**R**	S	S
A5	S	**R**	**R**	**R**	S	**R**	**R**	**R**	**R**	S	S
A6	**R**	S	**R**	**R**	S	**R**	**R**	**R**	S	**R**	**R**
A7	**R**	**R**	**R**	**R**	S	**R**	**R**	**R**	**R**	**R**	**R**

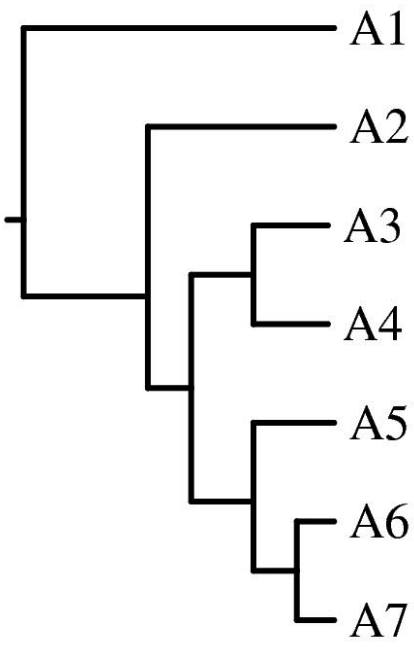
AK, Amikacin; CN, gentamycin; CIP, ciprofloxacin; PRL, piperacillin; CT, Colistin; TOB, Tobramycin; FEB, Cefepime; CAZ, ceftazidime; ATM, aztreonam; MEM, meropenem; IPM, imipenem ; S, sensitive; R, resistant.

The above table shows that the isolated *Pseudomonas aeruginosa* strains showed 7 antibiotic sensitivity patterns that were designated A1-A7. The antibiotic sensitivity patterns range from pattern A1 which is sensitive to all tested antibiotics to pattern A7 which is resistant to all tested antibiotics except colistin.

Table (15): Similarity Matrix computed with Dice coefficient for different observed antibiotic resistance patterns

	A1	A2	A3	A4	A5	A6	A7
A1	1	0.000	0.000	0.000	0.000	0.000	0.000
A2		1	0.286	0.571	0.400	0.364	0.308
A3			1	0.750	0.545	0.333	0.571
A4				1	0.727	0.333	0.571
A5					1	0.667	0.824
A6						1	0.889
A7							1

Fig. (14): Dendrogram of antibiotic resistance profiles for Pseudomonas isolates based on numerical analysis of the results of antibiotic susceptibility patterns.



D. RAPD ANALYSIS

All the *Pseudomonas aeruginosa* isolates were typable by RAPD method. The patterns consisted of 2 to 7 bands ranging from 200 bp to 2000 bp (fig. 15). The typing showed seven RAPD patterns (RAPDI-RAPDVII).

Fig. (15): Different Observed RAPD patterns for isolated *Pseudomonas aeruginosa* strains

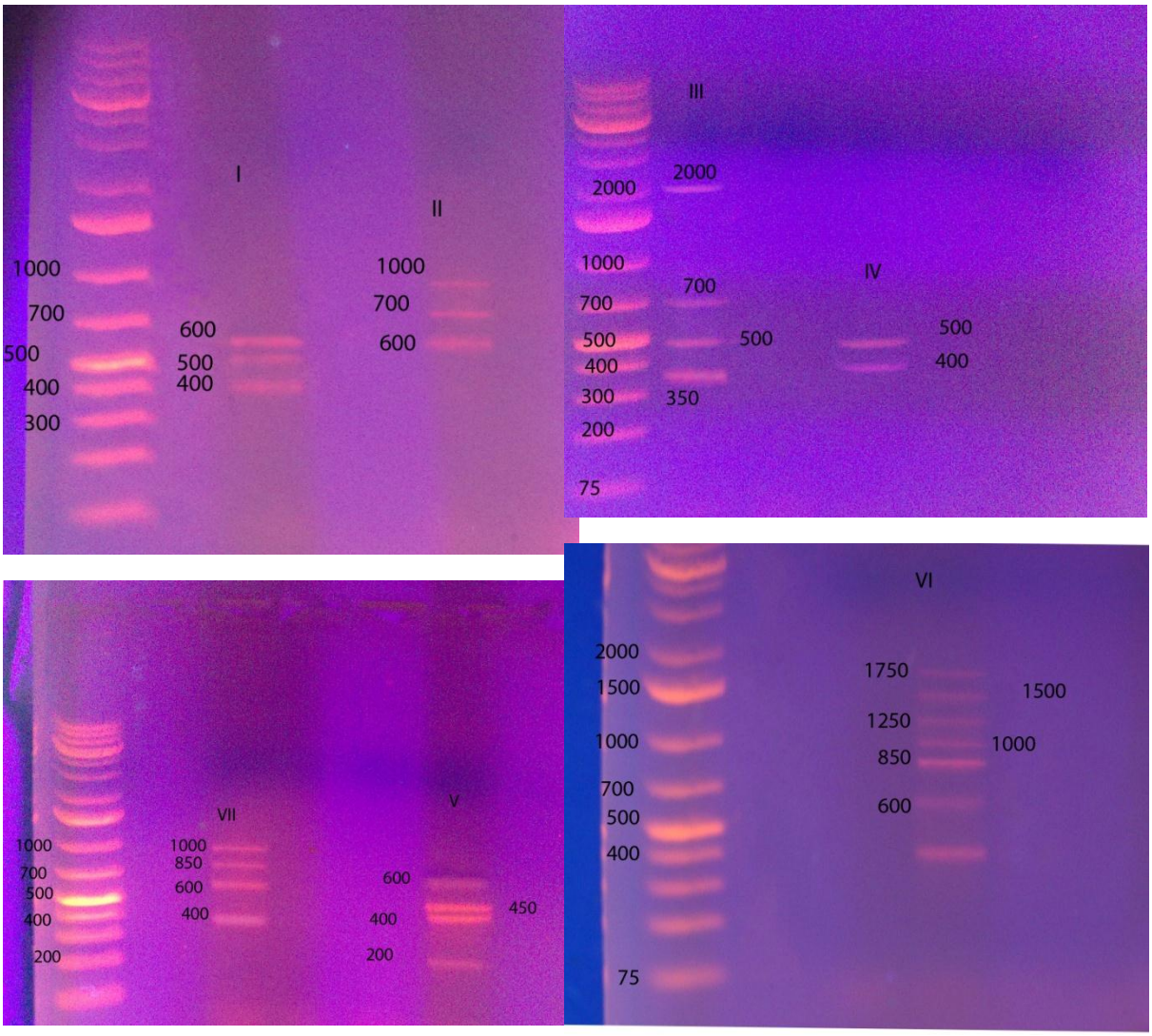


Table (16): Observed RAPD patterns for isolated *Ps. aeruginosa* strains

RAPD pattern	Observed bands (bp)												
	200	350	400	450	500	600	700	850	1000	1250	1500	1750	2000
I	0	0	1	0	1	1	0	0	0	0	0	0	0
II	0	0	0	0	0	1	1	0	1	0	0	0	0
III	0	1	0	0	1	0	1	0	0	0	0	0	1
IV	0	0	1	0	1	0	0	0	0	0	0	0	0
V	1	0	1	1	0	1	0	0	0	0	0	0	0
VI	0	0	1	0	0	1	0	1	1	1	1	1	0
VII	0	0	1	0	0	1	0	1	1	0	0	0	0

1 = present; 0 = absent

RAPD patterns were analyzed visually and the presence (1) or absence (0) of bands was recorded to generate the above binary table.

Table (17): Similarity Matrix computed with Dice coefficient for different observed RAPD patterns

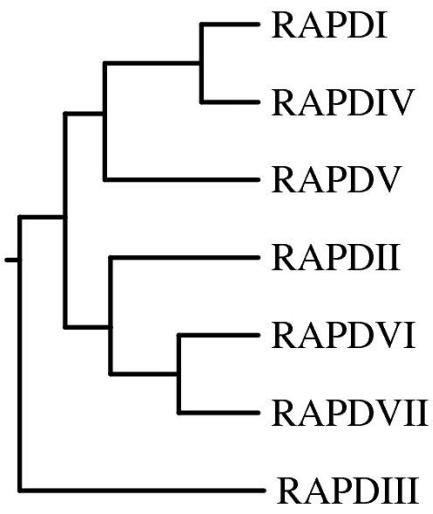
	RAPDI	RAPDII	RAPDIII	RAPDIV	RAPDV	RAPDVI	RAPDVII
RAPDI	1	0.333	0.286	0.800	0.571	0.400	0.571
RAPDII		1	0.286	0.000	0.286	0.400	0.571
RAPDIII			1	0.333	0.000	0.000	0.000
RAPDIV				1	0.333	0.222	0.333
RAPDV					1	0.364	0.500
RAPDVI						1	0.727
RAPDVII							1

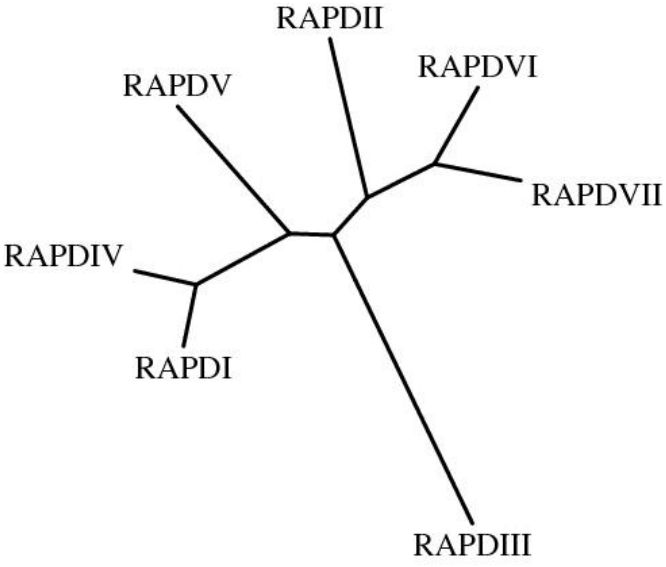
The similarity varied from 0 to 0.8 the larger the number the more correlation.

Highest correlation was between RAPDIV and RAPDI.

Fig. (16): RAPD patterns dendrogram

Dendrogram of RAPD genomic fingerprints of *P. aeruginosa isolates*. Percent similarity between patterns was calculated using Dice coefficients. The clustering pattern was generated using the UPGMA method.

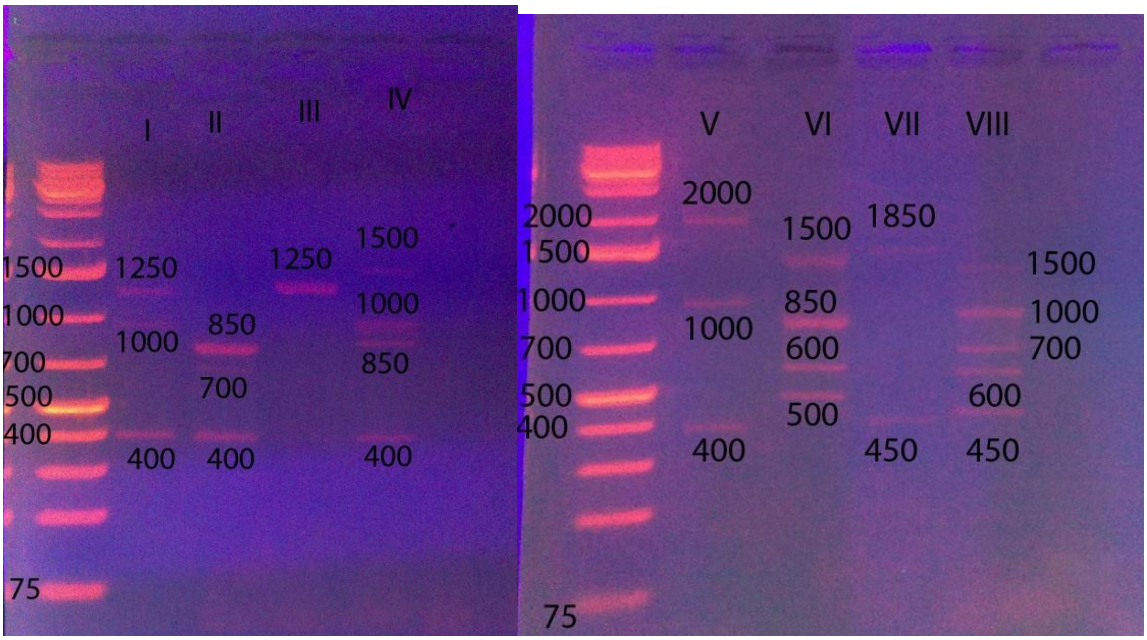




E. ERIC ANALYSIS

ERIC-PCR yielded 1 to 5 amplification bands. The size of amplified DNA bands ranged from 75 bp to 7000 bp (Fig. 17). All the 60 isolates were typable by this method. Eight ERIC patterns were obtained (ERICI-ERICVIII).

Fig. (17): Different Observed ERIC patterns for isolated *Pseudomonas aeruginosa* strains.



This figure shows the eight ERIC patterns that were observed. These patterns were designated ERICI-ERICVIII.

Table (18): Observed ERIC patterns for isolated *Ps. aeruginosa* stains

ERIC Pattern	Observed bands (kb)										
	400	450	500	600	700	850	1000	1250	1500	1850	2000
I	1	0	0	0	0	0	1	1	0	0	0
II	1	0	0	0	1	1	0	0	0	0	0
III	0	0	0	0	0	0	0	1	0	0	0
IV	1	0	0	0	0	1	1	0	1	0	0
V	1	0	0	0	0	0	1	0	0	0	1
VI	0	0	1	1	0	1	0	0	1	0	0
VII	0	1	0	0	0	0	0	0	0	1	0
VIII	0	1	0	1	1	0	1	0	1	0	0

1 = present; 0 = absent

RAPD patterns were analyzed visually and the presence or absence of bands was recorded to generate the above binary table.

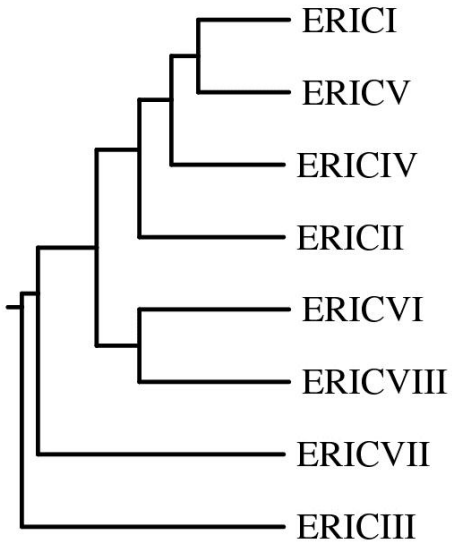
Table (19): Similarity Matrix computed with Dice coefficient for different observed EIRC PCR patterns

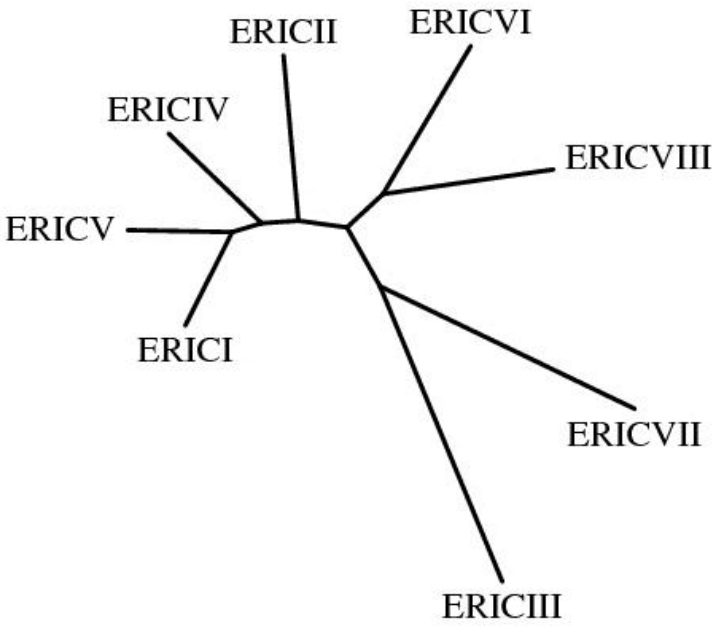
	ERICI	ERICII	ERICIII	ERICIV	ERICV	ERICVI	ERICVII	ERICVIII
ERICI	1	0.333	0.500	0.571	0.667	0.000	0.000	0.250
ERICII		1	0.000	0.571	0.333	0.286	0.000	0.250
ERICIII			1	0.000	0.000	0.000	0.000	0.000
ERICIV				1	0.571	0.500	0.000	0.444
ERICV					1	0.000	0.000	0.250
ERICVI						1	0.000	0.444
ERICVII							1	0.286
ERICVIII								1

This table compares the similarity between each pair of ERIC patterns. The similarity between different ERIC patterns ranged from 0 to 0.667.

Fig. (18) ERIC PCR dendrogram

Dendrogram of ERIC-PCR genomic fingerprints of *P. aeruginosa* isolates. Percent similarity between patterns was calculated using Dice coefficients. The clustering pattern was generated using the UPGMA method.





F. COMPARISON OF RAPD AND ERIC TYPING METHODS

Table (20): Numerical discriminatory index of ERIC, RAPD and Antibiogram.

	No. of different types	No. of strains belonging to the most numerous type	Numerical discriminatory index
ERIC	8	24	0.7955
RAPD	7	24	0.7706
Combined RAPD/ERIC	9	24	0.7977
Antibiogram	7	24	0.7232

The above table showed that ERIC typing method gave higher discriminatory index (0.7955) than RAPD (0.7706) and that the combination of both (0.7977) was superior to either alone. Antibiogram gave the lowest discriminatory index (0.7232).

G. EPIDEMIOLOGICAL ANALYSIS OF TYPING DATA

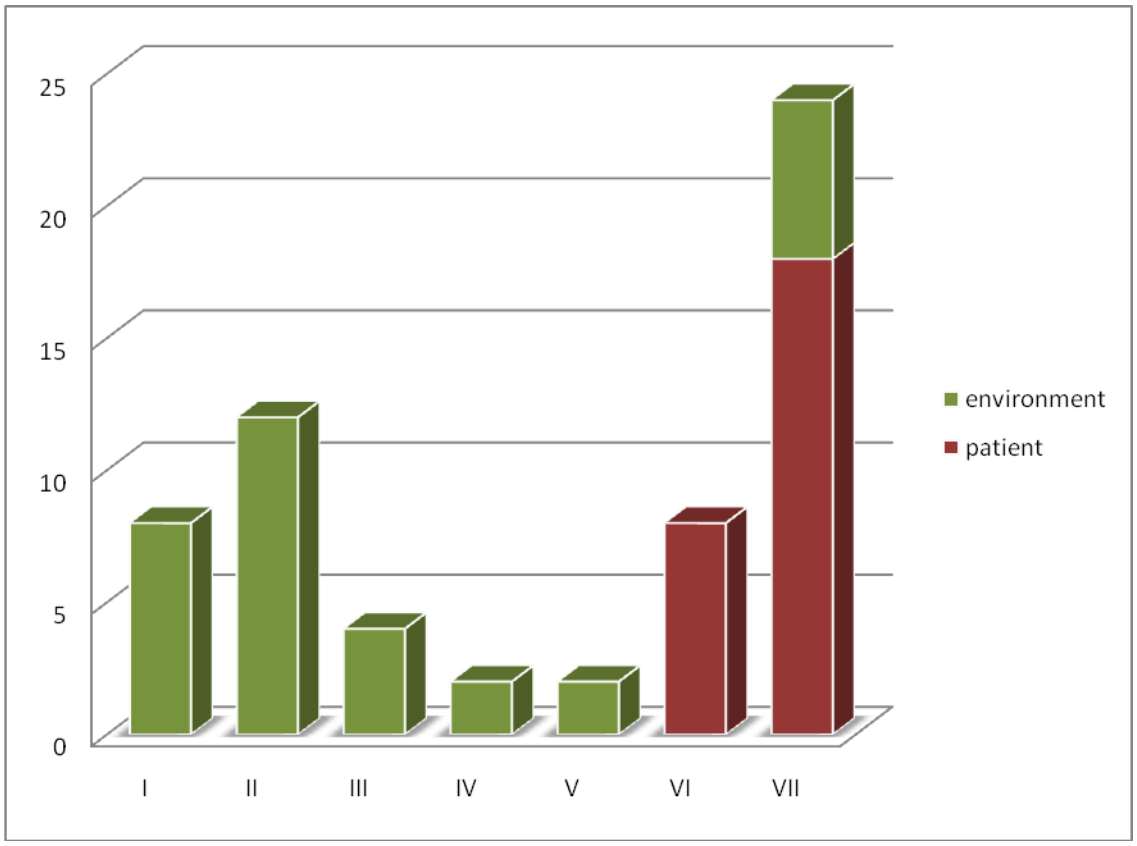
Table (21): List showing details of *Pseudomonas* isolates.

Isolates	Source	Antibiotic group	RAPD pattern	ERIC pattern
e51, e54	AV fluid reservoir	1	I	I
h08, h12	Hand	1	I	I
e38, e40, e43, e86	Water sink	1	I	I
e91, e87	Tray	1	II	II
e01, e03	Stethoscope	1	II	II
e44, e90	Suction apparatus tubing	1	II	II
e02, e24, e42, e53, e55, e79	Floor	1	II	III
e23, e89	Air conditioner	2	III	IV
e80, e92	Patient side drawers	3	III	IV
e74, e85	Ambu bags	4	IV	V
e22, e88	AV tubing	5	V	VI
p07s, p21s	Patient	5	VI	VI
p10u, p16u, p08u, p26u, p03u, p15u	Patient	6	VI	VII
e78, e52, e39, e04	Water tap/sink	7	VII	VIII
e41, e84	Suction apparatus tubing	7	VII	VIII
p01s, p28s, p01u, p28u, p07u, p21u, p09s, p25s, p17s, p22s, p04s, p14s,	Patient	7	VII	VIII

p18s, p04u, p14u, p18u, p05s, p12s	

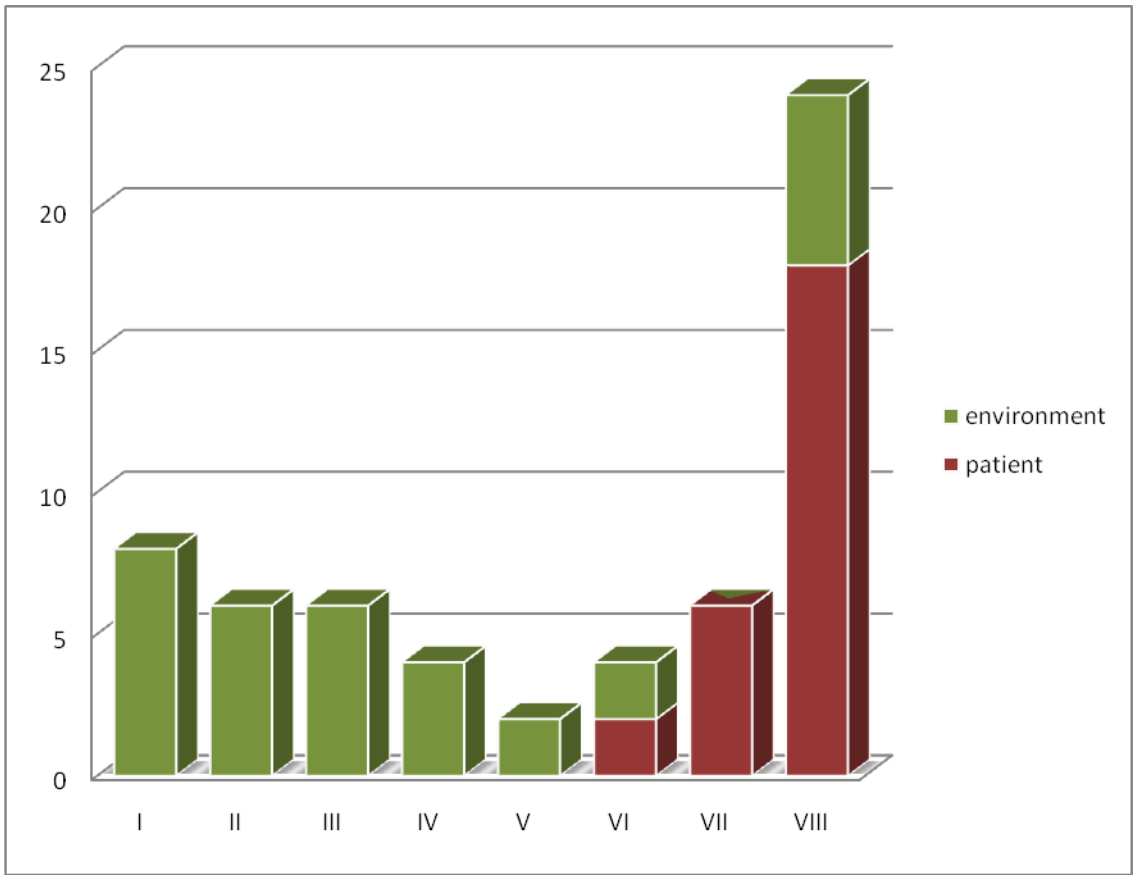
NB. MBL producing strains belong to antibiotic groups A6 & A7.

Fig.(19): Distribution of Pseudomonas isolates among various RAPD genotypes



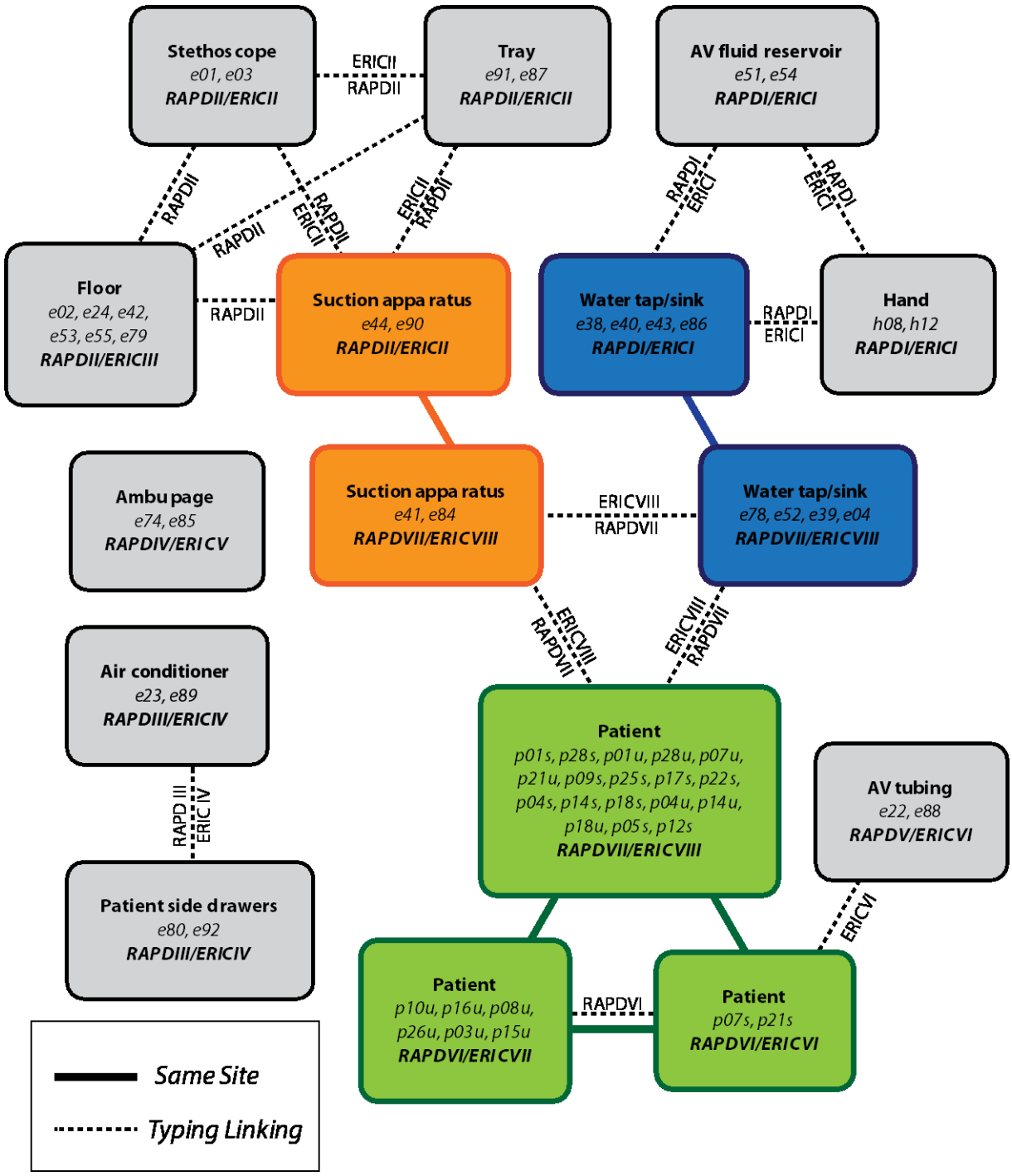
The above chart shows that patient isolates belong to RAPD genotypes VI and VII. Environmental isolates belongs to RAPD genotypes I, II, III, IV, V and VII. Genotype VII contains both environmental and patient isolates.

Fig.(20): Distribution of *Pseudomonas* isolates among various ERIC genotypes



The above chart shows that patient isolates belong to ERIC genotypes VI, VII and VIII. Environmental isolates belongs to RAPD genotypes I, II, III, IV, V, VI and VIII. Genotypes VI and VIII contain both environmental and patient isolates.

Fig. (21): Graphical analysis of epidemiologic relationships proved by molecular typing methods among different sites of *Pseudomonas* isolation



This figure shows that water-tap and suction apparatus has central role in the spread of *Pseudomonas aeruginosa* in the ICU.

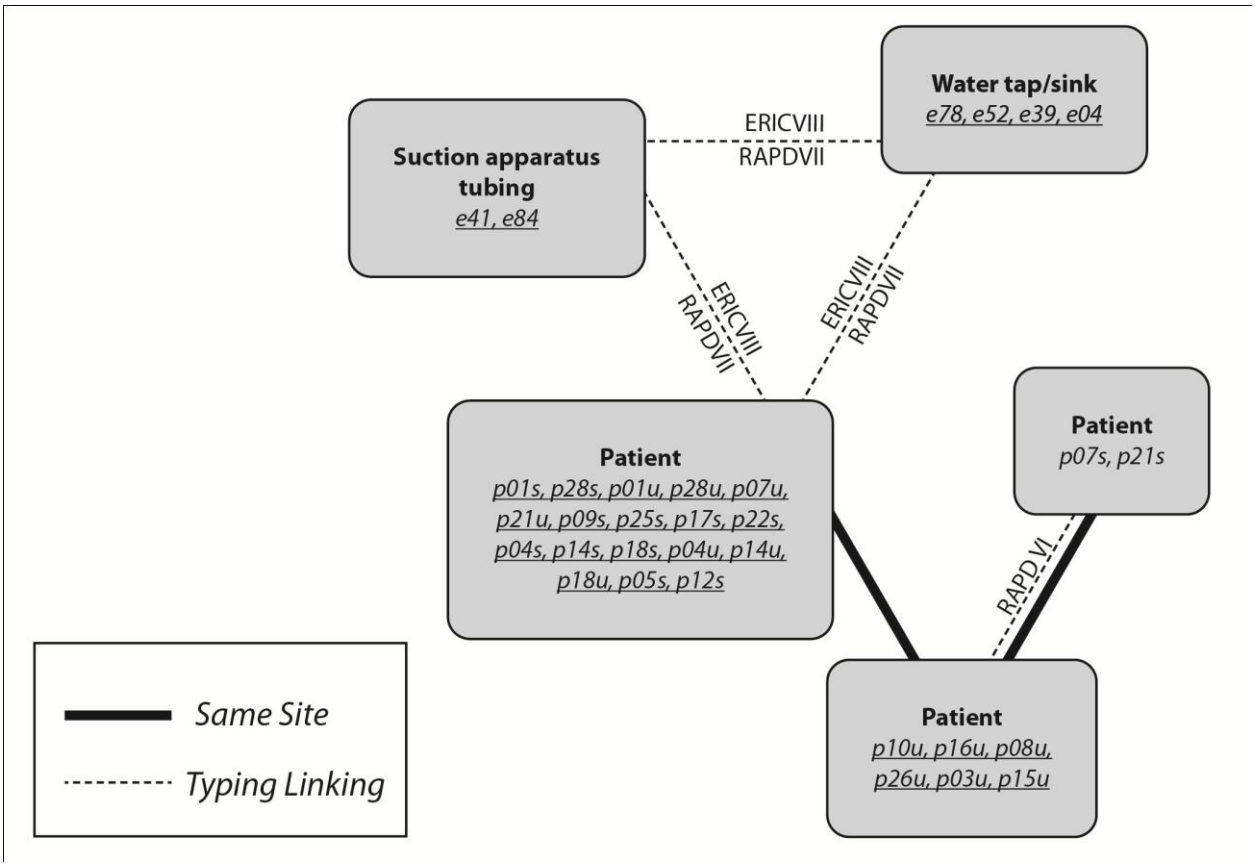
Water-tap has epidemiological relations with staff hands and artificial ventilation fluid reservoir by harboring strains that share the genotypes RAPD I and ERIC I.

Suction apparatus was linked to medical trays and stethoscope by harboring strains that share ERIC II and RAPD II genotypes. The stethoscope was linked to the floor by harboring strains that share the RAPDII genotype.

Both water-tap and suction apparatus were linked epidemiologically by harboring strains that share ERIC VIII and RAPD VII genotypes. And both had been linked to patient by harboring stains that share the ERIC VIII and RAPD VII genotypes.

Epidemiological linkage has been also proved between patient and AV tubing by harboring strains belonging to the ERIC VI genotype.

Fig. (22): Graphical analysis of epidemiological spread of MBL producing *Pseudomonas*



The above figure shows that patient MBL-producing strains are linked epidemiologically to water tap and suction apparatus tubing by harboring isolates that belong to the genotypes ERICVIII and RAPD VII.

Some MBL producing stains were linked to non MBL strains by sharing the RAPD VI genotypes.