RESULTS

This study was done on 35patients (fourteen males and twenty one females) admitted to intensive care unit of Benha University Hospital from December 2009 to September 2010. Their age ranged from 16– 86 years old. Those patients were suffered from either epilepsy, cerebrovascular stroke (CVS), renal failure, respiratory failure or chronic obstructive pulmonary disease (COPD) all patients were mechanically ventilated.

- Two tracheal aspirates were taken from each patient:

- First sample within 24 hour from intubation.
- Second sample after 72 hour from intubation

- Each tracheal aspirate sample was cultured on Robertson cooked meat medium to be transported immediately to the laboratory where the surface of the medium was covered with 1ml of sterile liquid paraffin and incubated for 24-48 hour. Subcultures were made on the selective media i.e anaerobic selective blood agar and MRS agar .The plates were incubated anaerobically using the anaerobic gas pack jar, for 48 hour at 37∘c .

- Antibiotic susceptibility was done using disc diffusion method by culturing the isolated organisms on 5% blood agar with Muller-Hinton agar base.

- Identification of isolated anaerobic organisms up to the species level was performed by using RapID-ANA II system.

The results of this study are represented in tables 7-32 and figures 3-23

Table (7): Distribution of study group (35 patients) according to age

	Number of CASES	Minimum age	Maximum age	Mean ± SD
Age(years)	35	16	86	57.63±15.033

	No of cases			%
Age groups	<40y	3		8.6
	40-60y	16		45.7
	>60y	16		45.7
	Total	35		100

SD: standard deviation

Figure (3): Distribution of study group (35 patients) according to age
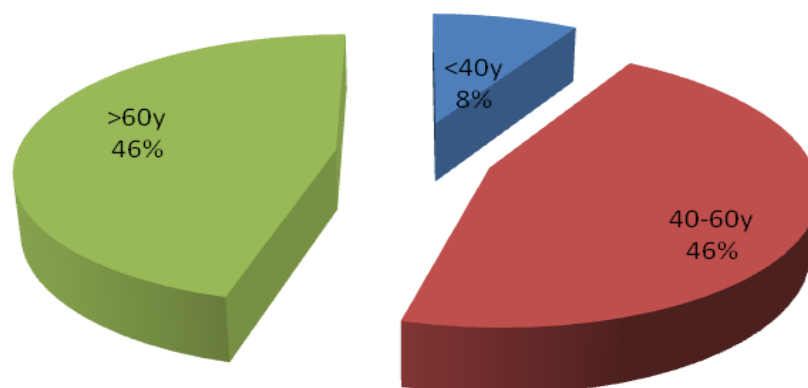
Table (8): Distribution of study group (35 patients) according to sex

		No of cases	%
sex	Female	21	60.0
	Male	14	40.0
	Total	35	100.0

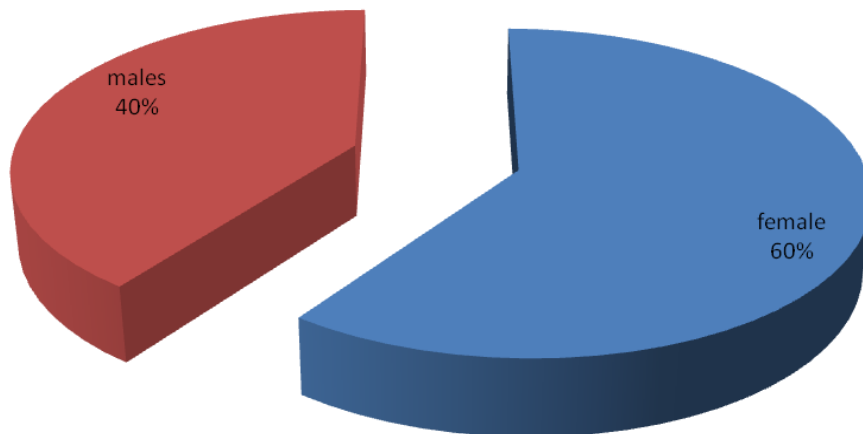*Figure (4): Distribution of study group (35 patients) according to sex*

Table (9): Distribution of study group (35 patients) as regards cause submitted to mechanical ventilation

Presenting condition	No. of patients
Cerebrovascular stroke	18
Renal failure	5
Respiratory failure	7
Septic shock	5
Total	35

N.B: Among 35 mechanically ventilated patients in this study , 27 patients were suffered from chronic diseases ;13 patients were suffered from diabetes mellitus and 18 patients were suffered from hypertension. (Four out of 27 patients with chronic diseases were suffered from both diabetes mellitus and hypertension).

Table (10): Comparison between results of anaerobic culture of tracheal aspirate samples within 24h of intubation as regards presence or absence of chronic disease

		Patient without chronic disease		Patient with chronic disease		Total		X^2	p*
		No of cases	%	No of cases	%	No of cases	%		
Within 24h	NEGATIVE culture	6	75.0%	19	70.4%	25	71.4%	0.1	>0.05
	POSITIVE culture	2	25.0%	8	29.6%	10	28.6%		
	Total	8	100.0%	27	100.0%	35	100.0%		

p*: Insignificant.

Table (10) shows that 2 (25.0%) out of 8 patients without chronic diseases and 8 (29.6%) out of 27 patients with chronic diseases gave positive result for anaerobic culture of tracheal aspirate samples within 24 hour of intubation. A difference which is insignificant.

Figure (5): Comparison between results of anaerobic culture of tracheal aspirate samples within 24h of intubation as regards presence and absence of chronic disease
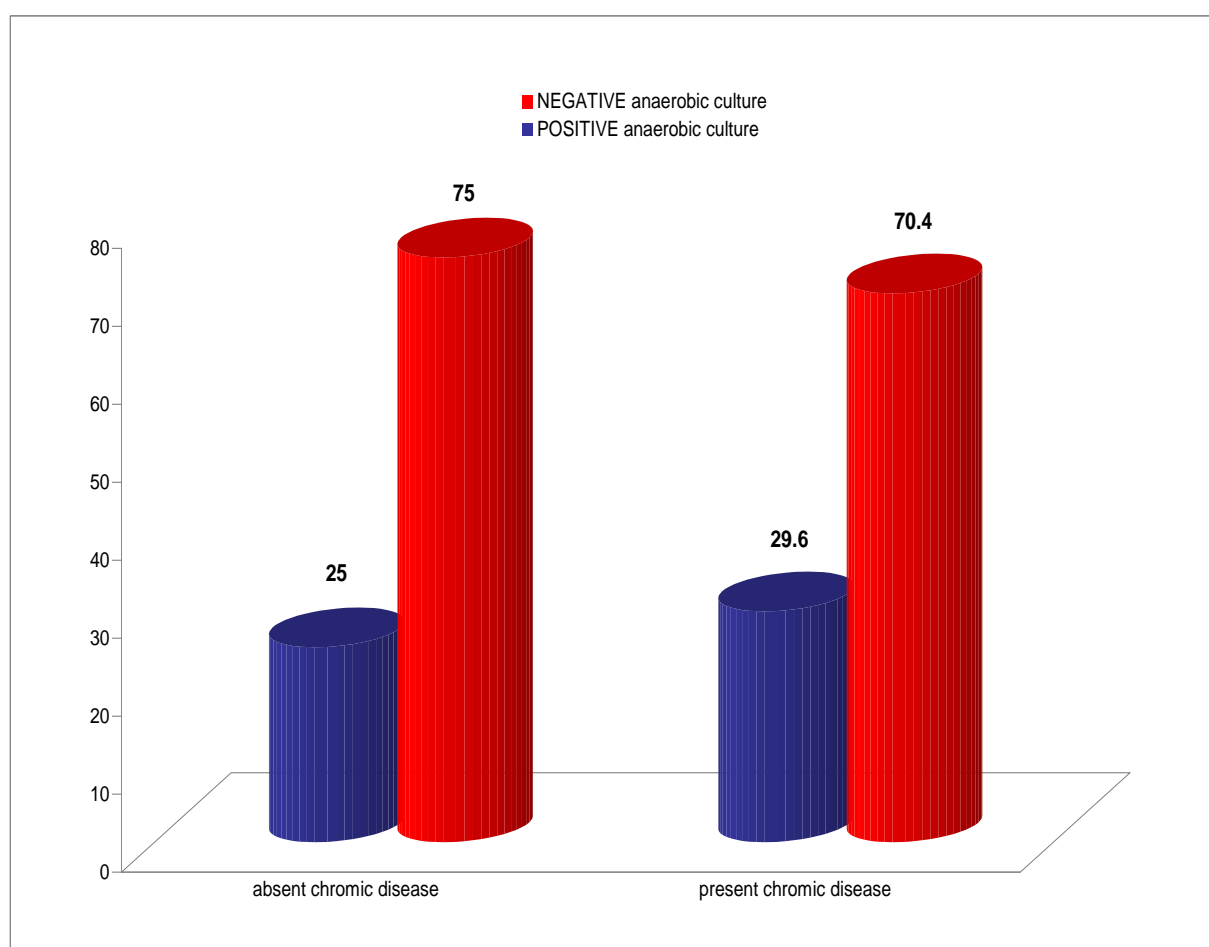
Table (11): Comparison between results of anaerobic culture of tracheal aspirate samples after 72h of intubation as regards presence or absence of chronic disease

		Patient without chronic disease		Patient with chronic disease		Total		X^2	p*
		No of cases	%	No of cases	%	No of cases	%		
After 72h	NEGATIVE CULTURE	3	12.5%	0	0.0%	3	8.6%	6.8	<0.05
	POSITIVE CULTURE	5	87.5%	27	100.0%	32	91.4%		
	Total	8	100.0%	27	100.0%	35	100.0%		

p*: Significant.

Table (11) shows that 5(**87.5%**) out of 8 patients without chronic diseases and all patients with chronic diseases (27 patients) gave positive result for anaerobic culture of tracheal aspirate samples after 72 hour of intubation. A difference which is significant.

Figure(6): Comparison between results of anaerobic culture of tracheal aspirate samples after 72h of intubation as regards presence and absence of Chronic disease
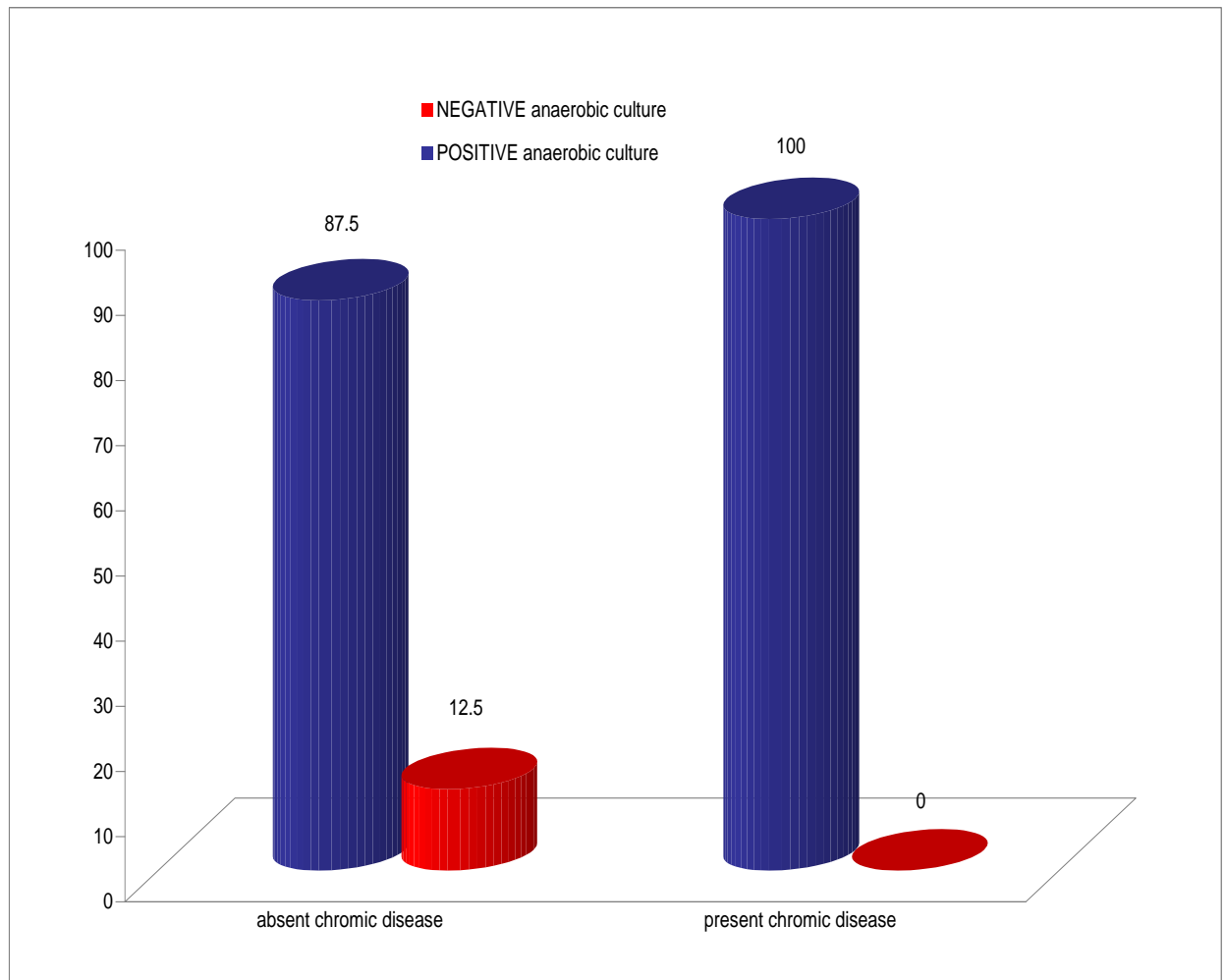
Table (12): Comparison between results of anaerobic culture of tracheal aspirate samples within 24h of intubation and after 72h of intubation as regards presence of Diabetes mellitus

	Positive culture		Negative culture		Total		X^2	p*
	No of cases	%	No of cases	%	No of cases	%		
1ˢᵗ sample Within24h	4	30.8%	9	69.2%	13	100.0%	10.9	<0.05
2ⁿᵈsample After 72h	13	100.0%	0	0.00%	13	100.0%		

p*: Significant.

Table (12)shows that 4 (30.8%) out of 13 patients with diabetes mellitus gave positive result of anaerobic culture of tracheal aspirate samples taken within 24 hour of intubation , while all of them gave positive culture from samples taken after 72 hour of intubation . A difference which is significant.

Figure(7): Comparison between results of anaerobic culture of tracheal aspirate samples within 24h(1st sample) of intubation and after 72h (2nd sample) of intubation as regards presence of Diabetes mellitus
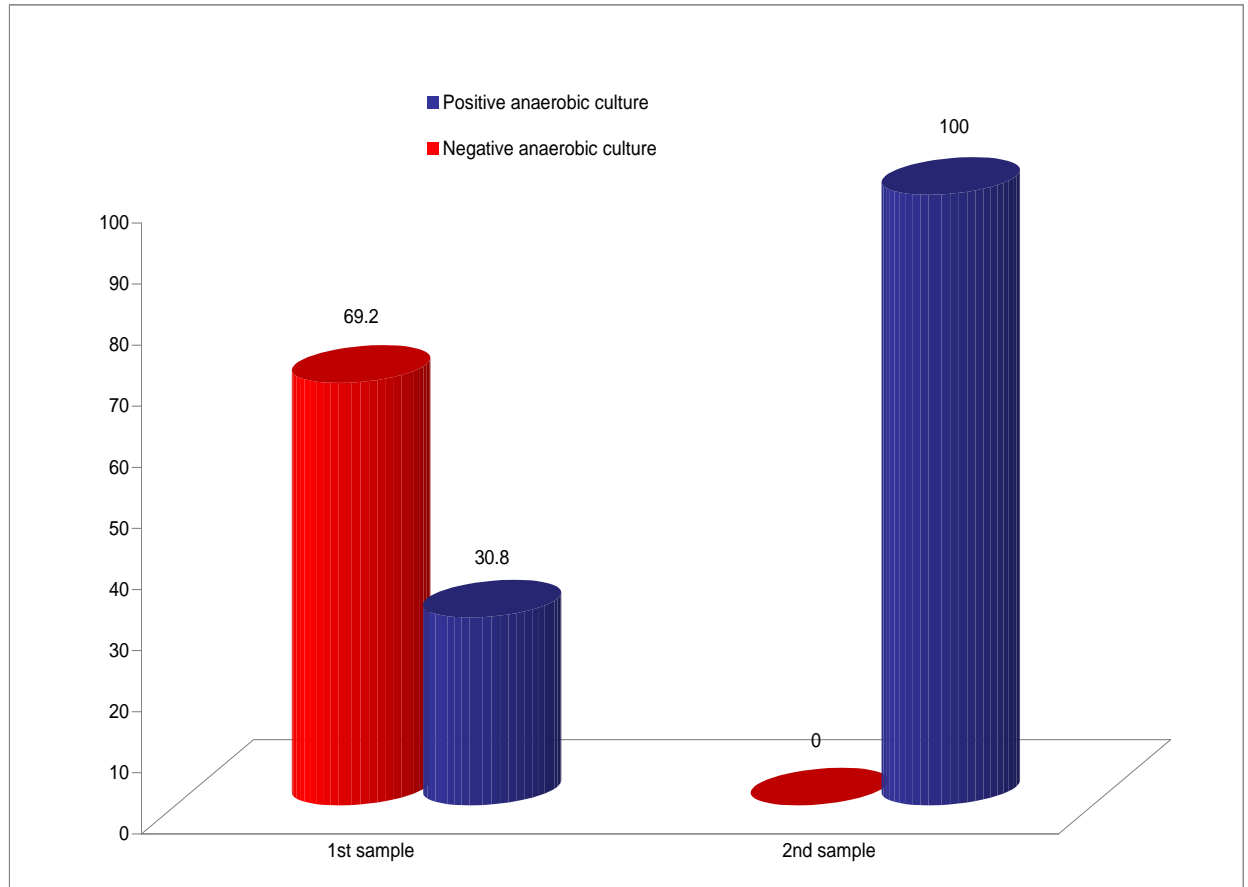
Table (13): Comparison between results of anaerobic culture of tracheal aspirate samples within 24h of intubation and after 72h of intubation as regards presence of Hypertension

	Positive culture		Negative culture		Total		X^2	p*
	No of cases	%	No of cases	%	No of cases	%		
1st sample Within24h	6	33.3%	12	66.7%	18	100.0%	15.1	<0.05
2nd sample After 72h	18	100.0%	0	0.00%	18	100.0%		

p*: Significant.

Table (13) shows that 6 (**33.3%**)out of 18 patients with Hypertension gave positive result of anaerobic culture of tracheal aspirate samples taken within 24 hour of intubation , while all of them gave positive culture from samples taken after 72 hour of intubation . A difference which is significant.

Figure(8): Comparison between results of anaerobic culture of tracheal aspirate samples within 24h(1st sample) of intubation and after 72h (2nd sample) of intubation as regards presence of Hypertension
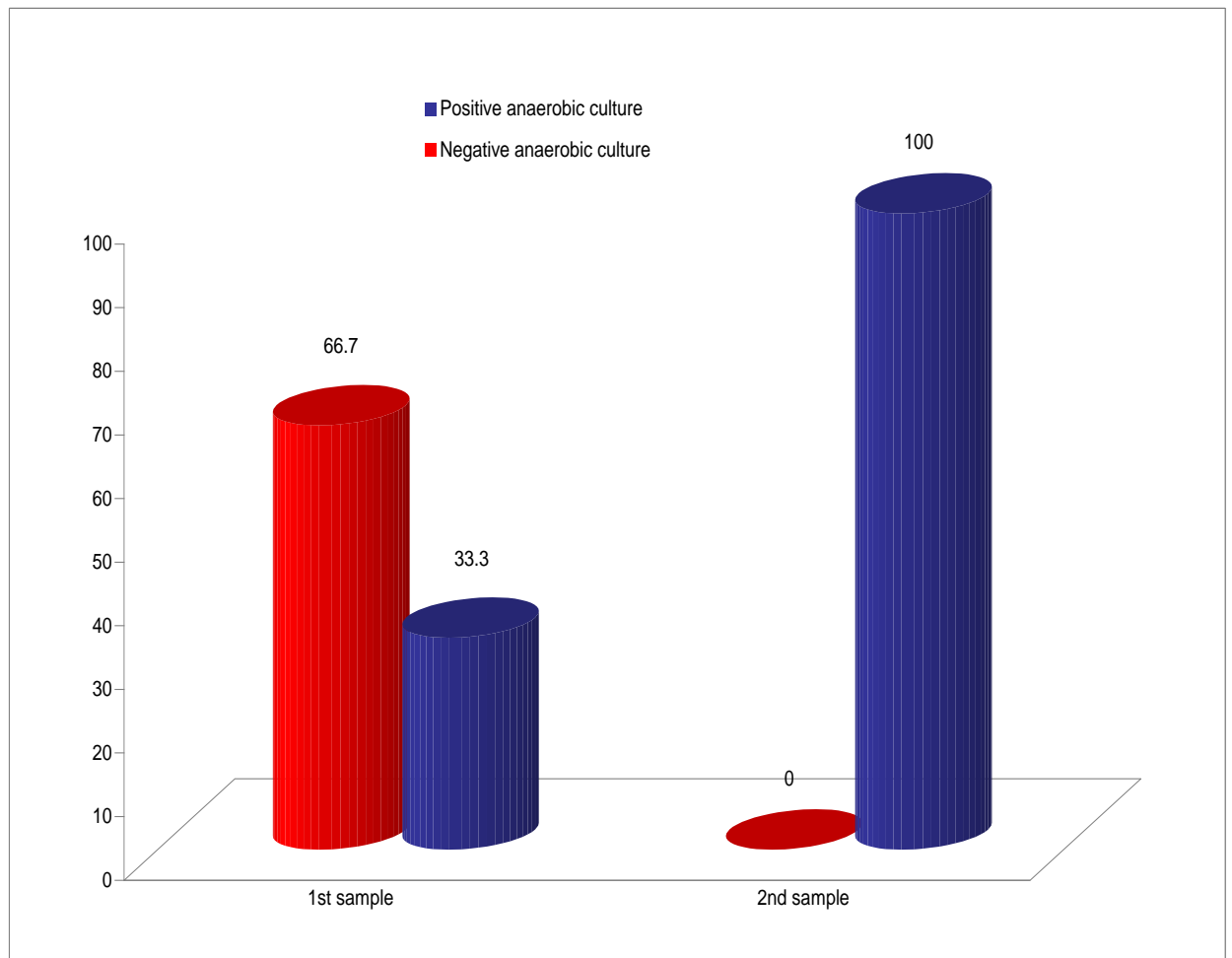
Table (14): Comparison between results of anaerobic culture of tracheal aspirate samples within 24h of intubation as regards each presenting condition

presenting condition	Positive culture		Negative culture		Total	
	No of cases	%	No of cases	%	No of cases	%
Cerebrovascular stroke	5	27.8%	13	72.2%	18	100.0%
Renal failure	1	20%	4	80%	5	100.0%
Respiratory failure	2	28.6%	5	71.4%	7	100.0%
Shock	2	40%	3	60.0%	5	100.0%
TOTAL	10	28.57%	25	71.43%	35	100.0%

Table (15): Comparison between results of anaerobic culture of tracheal aspirate samples after 72h of intubation as regards each presenting disease

presenting condition	Positive culture		Negative culture		Total	
	No of cases	%	No of cases	%	No of cases	%
Cerebrovascular stroke	18	100.0%	0	0.00%	18	100.0%
Renal failure	4	80%	1	20%	5	100.0%
Respiratory failure	7	100.0%	0	0.00%	7	100.0%
shock	3	60.0%	1	40.0.%	5	100.0%
TOTAL	32	*91.43%*	3	*8.57%*	35	100.0%

Table (16): Comparison between results of anaerobic culture of tracheal aspirate samples within 24h of intubation and after 72h of intubation as regards Cerebrovascular stroke

	Positive culture		Negative culture		Total		X^2	p*
	No of cases	%	No of cases	%	No of cases	%		
1st sample within24h	5	27.8%	13	72.2%	18	100.0%	17.3	<0.001
2nd sample after 72h	18	100.0%	0	0.00%	18	100.0%		

p*: Highly significant.

Table (16) shows that 5 (27.8%) out of 18 patients with Cerebrovascular stroke gave positive anaerobic culture from 1st sample , while all patients (18 patients) with Cerebrovascular stroke gave positive anaerobic culture from samples taken after 72 hour of intubation . A difference which is highly significant.

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Figure(9): Comparison between results of anaerobic culture of tracheal aspirate samples within 24h(1ˢᵗ sample) of intubation and after 72h(2ⁿᵈ sample) of intubation as regards Cerebrovascular stroke
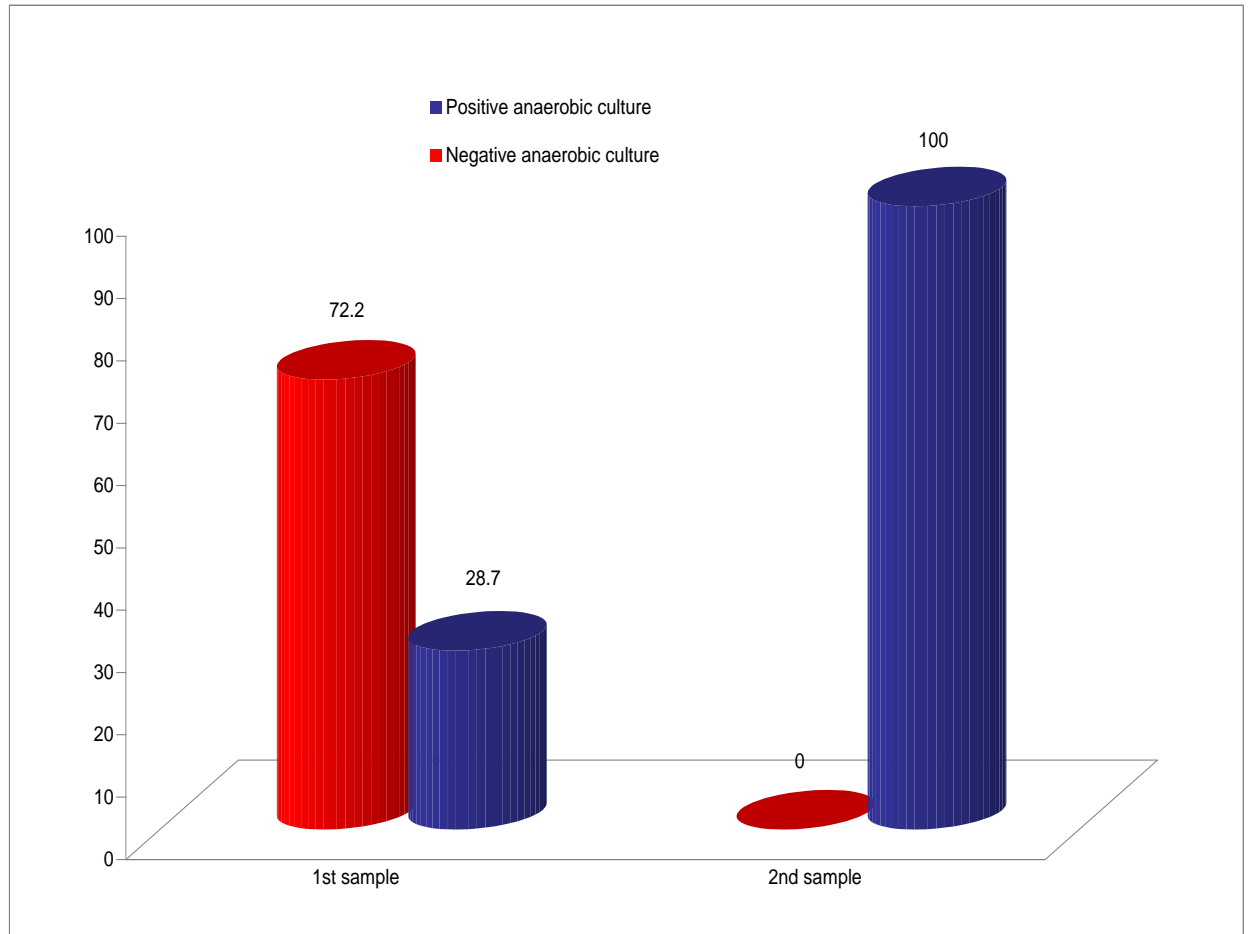
Table (17): Comparison between results of anaerobic culture of tracheal aspirate samples within 24h of intubation and after 72h of intubation as regards **renal failure**

	Positive culture		Negative culture		Total		X^2	p
	No of cases	%	No of cases	%	No of cases	%		
1st sample within24h	1	20%	4	80%	5	100.0%	3.6	>0.05
2nd sample after 72h	4	80%	1	20%	5	100.0%		

p*: Insignificant.

Table (17) shows that one (20%) out of 5 patients with renal failure gave positive anaerobic culture from 1st sample while 4 (80%) out of 5 patients with renal failure gave positive anaerobic culture from samples taken after 72 hour of intubation .A difference which is insignificant.

Figure(10): Comparison between results of anaerobic culture of tracheal aspirate samples within 24h(1ˢᵗ sample) of intubation and after 72h (2ⁿᵈ sample) of intubation as regards **renal failure**
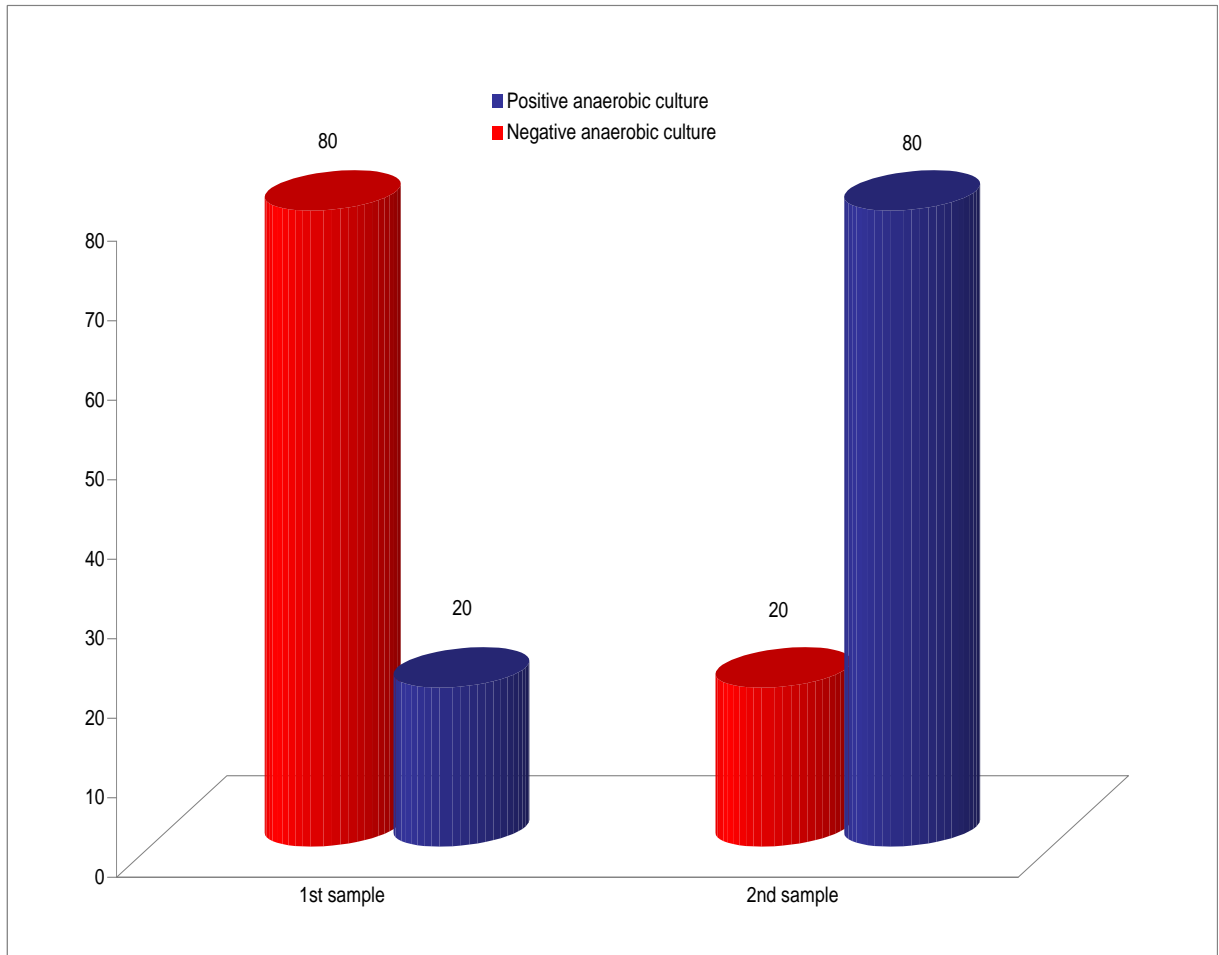
Table (18): Comparison between results of anaerobic culture of tracheal aspirate samples within 24h of intubation and after 72h of intubation as regards **respiratory failure**

	Positive culture		Negative culture		Total		X^2	p
	No of cases	%	No of cases	%	No of cases	%		
1st sample within24h	2	28.6%	5	71.4%	7	100.0%	7.8	<0.05
2nd sample after72h	7	100.0%	0	0.00%	7	100.0%		

p*: Significant.

Table (18) shows that 2 (28.6%) out of 7 patients with respiratory failure gave positive anaerobic culture from 1st sample while all patients(7 patients) with respiratory failure gave positive anaerobic culture from samples taken after 72 hour of intubation. A difference which is significant.

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Figure (11): Comparison between results of anaerobic culture of tracheal aspirate samples within 24h (1st sample) of intubation and after 72h (2nd sample) of intubation as regards **respiratory failure**
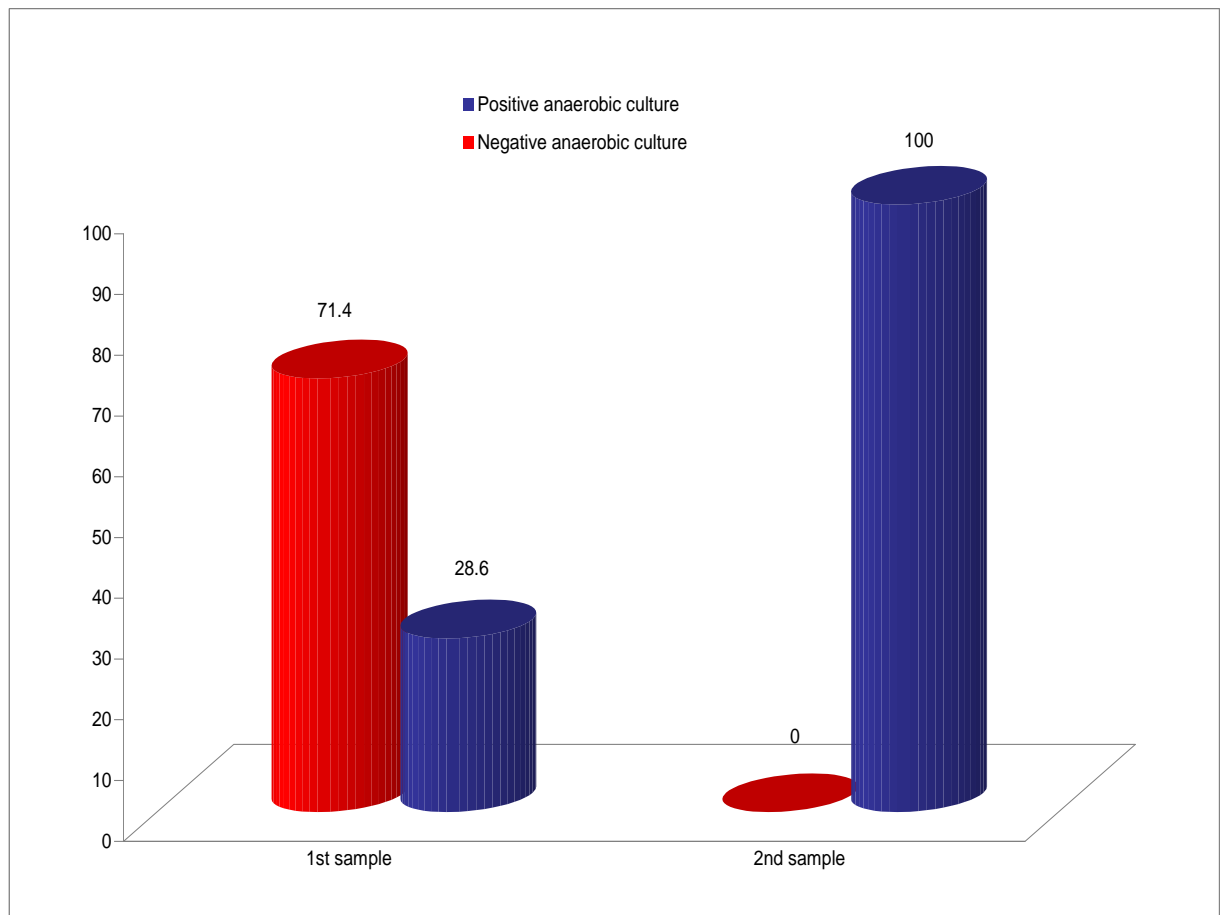
Table (19): Comparison between results of anaerobic culture of tracheal aspirate samples within 24h of intubation and after 72h of intubation as regards **shock**

	Positive culture		Negative culture		Total		X^2	p
	No of cases	%	No of cases	%	No of cases	%		
1st sample within24h	2	40%	3	60%	5	100.0%	0.4	>0.05
2nd sample after72h	3	60%	2	40%	5	100.0%		

p*: Insignificant.

Table (19) shows that 2 (40%) out of 5patients with shock gave positive anaerobic culture from 1st sample while 3 (60%) out of 5 patients with shock gave positive anaerobic culture from samples taken after 72 hour of intubation . A difference which is insignificant.

Figure(12): Comparison between results of anaerobic culture of tracheal aspirate samples within 24h(1st sample) of intubation and after 72h (2nd sample) of intubation as regards **septic shock**
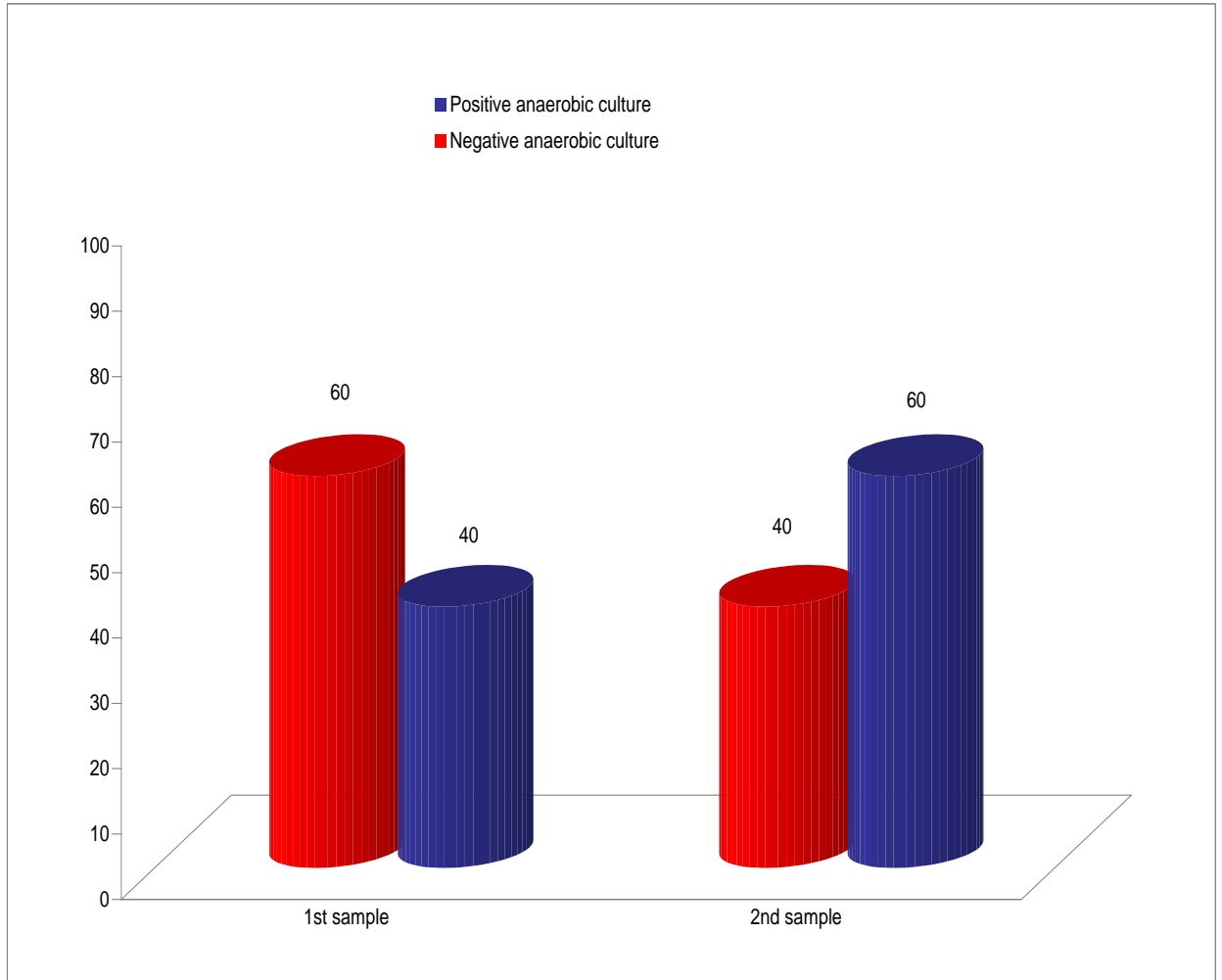
Table (20): Distribution of study group according to result of anaerobic culture of tracheal aspirate samples within 24h of intubation

		No of cases	%
within 24h	NEGATIVE CULTURE	25	71.4
	POSITIVE CULTURE	10	28.6
	Total	35	100.0

Table (20) shows that 10(28.6%) out of 35 patients gave positive result for anaerobic culture from samples taken within 24 hour of intubation.

Figure (13): Distribution of study group according to result of anaerobic culture of tracheal aspirate samples within 24h of intubation
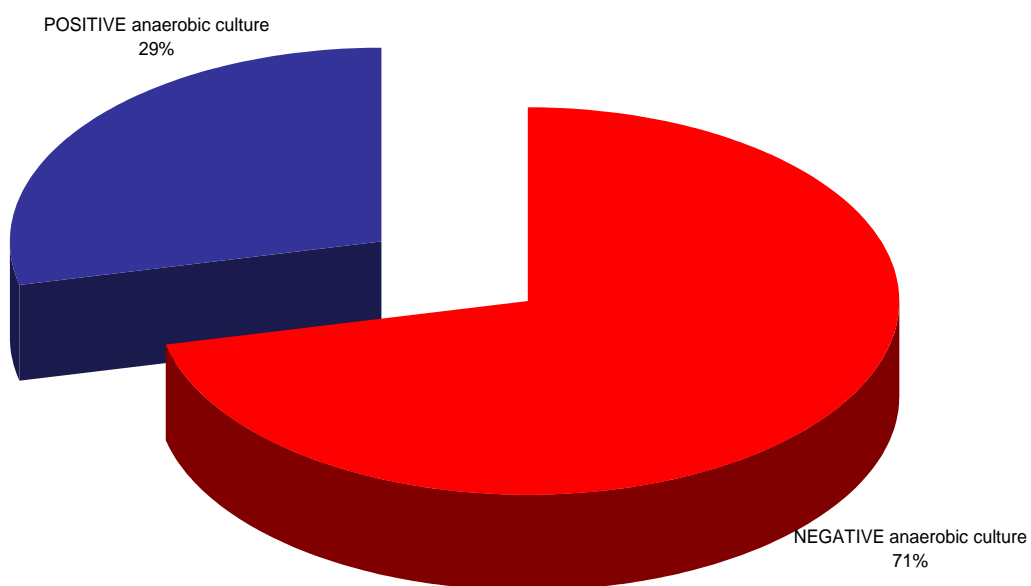

POSITIVE anaerobic culture
29%

NEGATIVE anaerobic culture
71%

Table (21): Distribution of study group according to result of anaerobic culture of tracheal aspirate samples after72h of intubation

		No of cases	%
after 72h	NEGATIVE CULTURE	3	8.6
	POSITIVE CULTURE	32	91.4
	Total	35	100.0

Table (21) shows that 32(91.4%) out of 35 patients gave positive result for anaerobic culture from samples taken after 72 hour of intubation.

Figure (14): Distribution of study group according to result of anaerobic culture of tracheal aspirate samples after72h of intubation
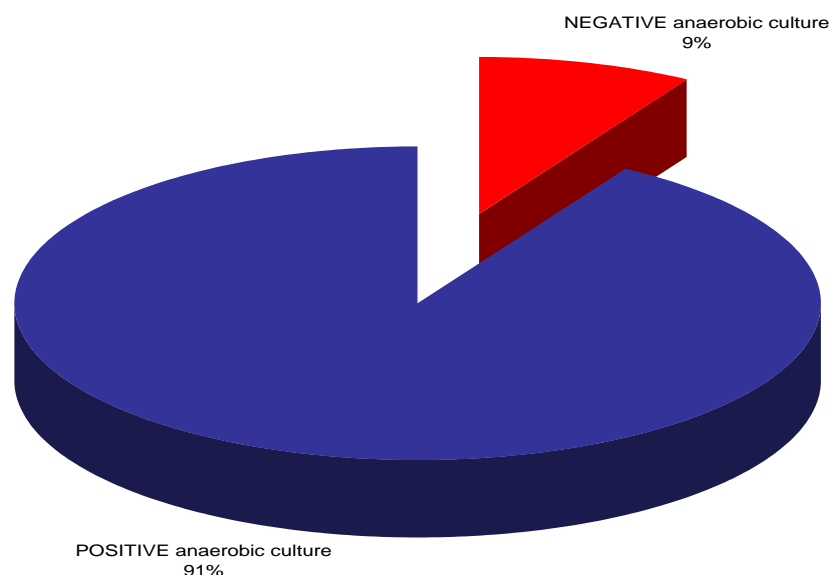
Table (22): Comparison between results of anaerobic culture of tracheal aspirate samples within 24h of intubation and after 72h of intubation

	No of positive cases	%
Within 24h	10	28.6
after 72h	32	91.4
Z	5.4	
p*	<0.001	

P* : Highly significant.

Table (22) shows that there is a highly significant difference in incidence of anaerobic isolates after prolonged intubation(72h).

Table (23): Isolated anaerobic organisms of the ten intubated patients in ICU who gave positive anaerobic culture from tracheal aspirate samples within 24h of intubation

Isolated organisms	No of isolates	%
Bacteroides	6	23.1%
Lactobacilli	8	30.8%
Peptostreptococcus	9	34.6%
Clostridium	3	11.5%
Total No. of isolates	26	100.0%

Table (24): Details of isolated anaerobic organisms of the ten intubated patients in ICU who gave positive anaerobic culture from tracheal aspirate samples within 24h of intubation

Case No.	Presenting condition	Isolated organisms	Total No.
1	Cerebrovascular stroke.	Bacteroides, Peptostreptococcus, Clostridium	3
2	Cerebrovascular stroke.	Peptostreptococcus, Bacteroides	2
3	Cerebrovascular stroke.	Clostridium, Lactobacilli,	2
4	Cerebrovascular stroke.	Bacteroides,Lactobacill Peptostreptococcus	3
5	Cerebrovascular stroke.	Peptostreptococcus,Clostridium	2
6	Renal failure.	Bacteroides,Lactobacilli, Peptostreptococcus	3
8	Respiratory failure.	Bacteroides,Lactobacilli Peptostreptococcus	3
9	Septic shock.	Peptostreptococcus, ,Lactobacilli	2
10	Septic shock.	Bacteroides,Lactobacilli, Peptostreptococcus	3
Total No of isolates			26

Table (25): Isolated anaerobic organisms of the thirty two intubated patients in ICU who gave positive anaerobic culture from tracheal aspirate samples after 72h of intubation

Isolated organisms	No of cases	%	Isolated organisms
Bacteroides	6	5.8%	Bacteroides
Prevotella	16	15.5%	Prevotella
Fusobacterium	16	15.5%	Fusobacterium
Lactobacilli	25	24.3%	Lactobacilli
Peptostreptococcus	20	19.5%	Peptostreptococcus
Veillonella	12	11.6%	Veillonella
Clostridium	8	7.8%	Clostridium
Total No. of isolates	103	1oo.o%	Total No. of isolates

Table (26): Details of isolated anaerobic organisms of the thirty two intubated patients in ICU who gave positive anaerobic culture from tracheal aspirate samples after 72h of intubation

Case No.	Presenting condition	Isolated organisms	Total No.
1	Cerebrovascular stroke.	Bacteroides,Prevotella,Fusobacterium Peptostreptococcus	4
2	Cerebrovascular stroke.	Bacteroide,Lactobacilli, Peptostreptococcus , Clostridium	4
3	Cerebrovascular stroke.	Prevotella,Fusobacterium ,Lactobacilli , Peptostreptococcus	4
4	Cerebrovascular stroke.	Bacteroides,Lactobacilli Peptostreptococcus, Clostridium	4
5	Cerebrovascular stroke.	Prevotella,Fusobacterium Peptostreptococcus, Veillonella	4
6	Cerebrovascular stroke.	Lactobacilli Veillonella, Bacteroides	3
7	Cerebrovascular stroke.	Prevotella,Fusobacterium ,Lactobacilli	3
8	Cerebrovascular stroke.	Lactobacilli,Peptostreptococcus, Veillonella, Bacteroides	4
9	Cerebrovascular stroke.	Prevotella,Fusobacterium, Peptostreptococcus	3
10	Cerebrovascular stroke.	Bacteroides, Lactobacilli ,Veillonella	3
11	Cerebrovascular stroke.	Lactobacilli,Peptostreptococcus ,Veillonella, Clostridium	4
12	Cerebrovascular stroke.	Prevotella,Fusobacterium, Lactobacilli	3
13	Cerebrovascular stroke.	Lactobacilli,Peptostreptococcus ,Veillonella	3
14	Cerebrovascular stroke.	Prevotella,Fusobacterium ,Lactobacilli, Veillonella	4
15	Cerebrovascular stroke.	Lactobacilli,Peptostreptococcus, Veillonella , Clostridium	4
16	Cerebrovascular stroke.	Lactobacilli,Peptostreptococcus Veillonella	3
17	Cerebrovascular stroke.	Fusobacterium , Lactobacilli	2
18	Cerebrovascular stroke.	Lactobacilli,Peptostreptococcus,Veillonella	3
19	Renal failure.	Prevotella ,Fusobacterium, Clostridium	3
20	Renal failure.	Clostridium, Prevotella ,Peptostreptococcus	3
21	Renal failure.	Prevotella, Lactobacilli, Peptostreptococcus, Clostridium	4
22	Renal failure.	Prevotella, Fusobacterium ,	3

		Peptostreptococcus	
23	Respiratory failure.	Fusobacterium , Lactobacilli	**2**
24	Respiratory failure.	Prevotella, Lactobacilli, Peptostreptococcus	**3**
25	Respiratory failure.	Prevotella, Fusobacterium, Peptostreptococcus	**3**
26	Respiratory failure.	Lactobacilli , Peptostreptococcus, Veillonella	**3**
27	Respiratory failure.	Fusobacterium , Lactobacilli	**2**
28	Respiratory failure.	Lactobacilli, Veillonella, Clostridium	**3**
29	Respiratory failure.	Prevotella,Fusobacterium, Lactobacilli,Peptostreptococcus	**4**
30	Septic shock.	Fusobacterium , Lactobacilli	**2**
31	Septic shock.	Prevotella , Lactobacilli, Peptostreptococcus	**3**
32	Septic shock.	Prevotella, Fusobacterium , Lactobacilli	**3**
Total No. of isolates			**103**

Table (27): Species of anaerobic organisms isolated from 72h tracheal aspirate samples of intubated patients in ICU

	No	**Species**
Bacteroides	**6**	**Bacteroides fragilis**
Prevotella	**7**	**P.melaninogenica**
	5	**P.oris**
	4	**P.oralis group**
Fusobacterium	**16**	**F. nucleatum**
Lactobacilli	**16**	**L.acidophillus**
	9	**L.catenaforme**
Peptostreptococcus	**9**	**P.anaerobius**
	7	**P.hydrogenalis**
	4	**P.micros**
Veillonella	**12**	**Veillonella spp.**
Clostridium	**8**	**Clostridium spp.**

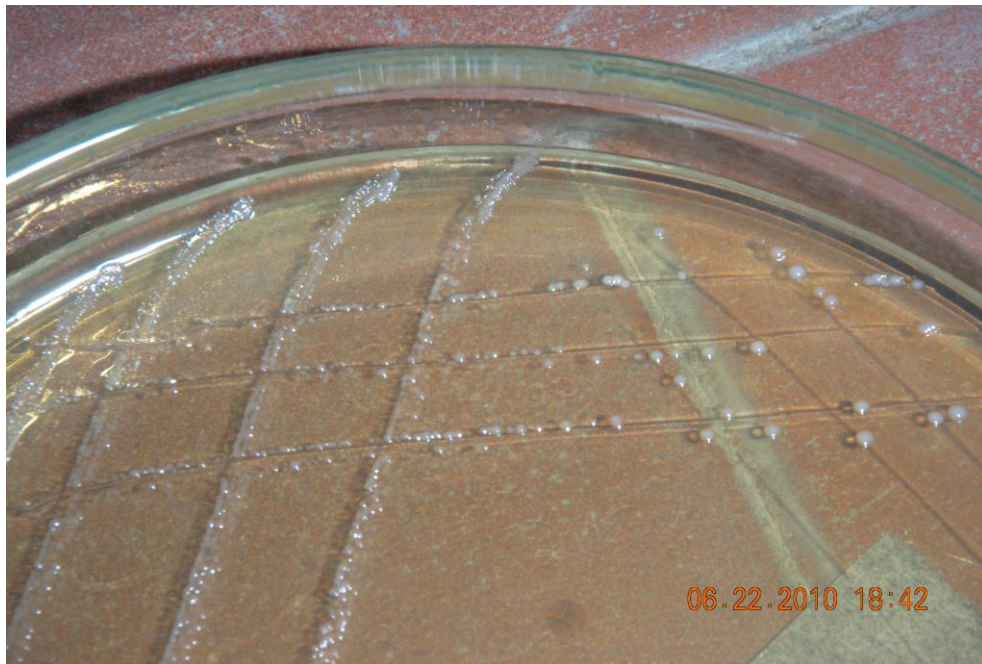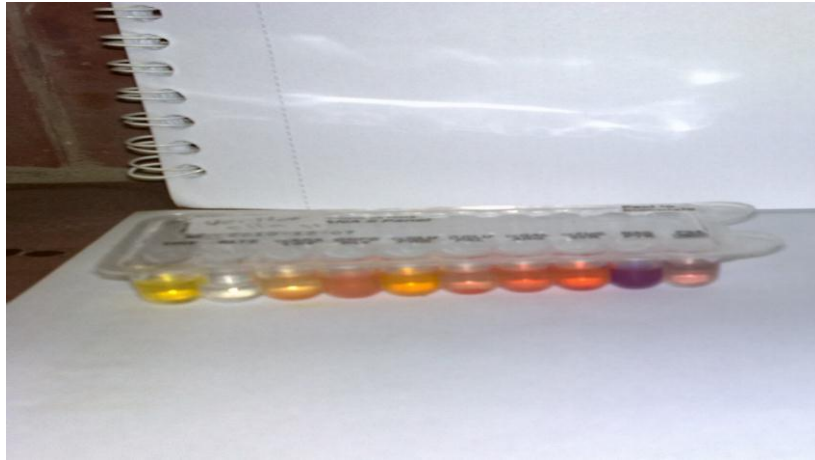*Figure (15):* MRS agar show *lactobacillius* culture .

Figure (16): Selective Blood agar show fusobacterium culture.

Figure (17): RapID II ANA System show *Veillonella* SPP.

Figure(18): RapID II ANA System show Prevotella oris SPP.

Figure(19): RapID II ANA System show *Peptostreptoccal anaerobius* SPP.

Table (28): Comparison between results of anaerobic culture of tracheal aspirate samples within 24h of intubation according to age groups

		Age								X^2	p*
		<40y		40- 60 y		> 60 y		Total			
		No of cases	%	No of cases	%	No of cases	%	No of cases	%		
within 24h	NEGATIVE CULTURE	2	66.7%	15	93.8%	8	50%	25	71.4%	7.5	<0.05
	POSITIVE CULTURE	1	33.3%	1	6.2%	8	50%	10	28.6%		
	Total	3	1oo%	16	100%	16	100%	35	100.0%		

P* :Significant.

Table (26) shows that one (33.3%) out of 3patients under 40 years old ,one (6.2%) out of 16 patients from 40-60 years old and 8 (50%) out of 16 above 60 years old gave positive anaerobic culture from samples taken within 24 hour of intubation. A difference which is significant.

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Figure(20): Comparison between results of anaerobic culture of tracheal aspirate samples within 24h of intubation according to age groups
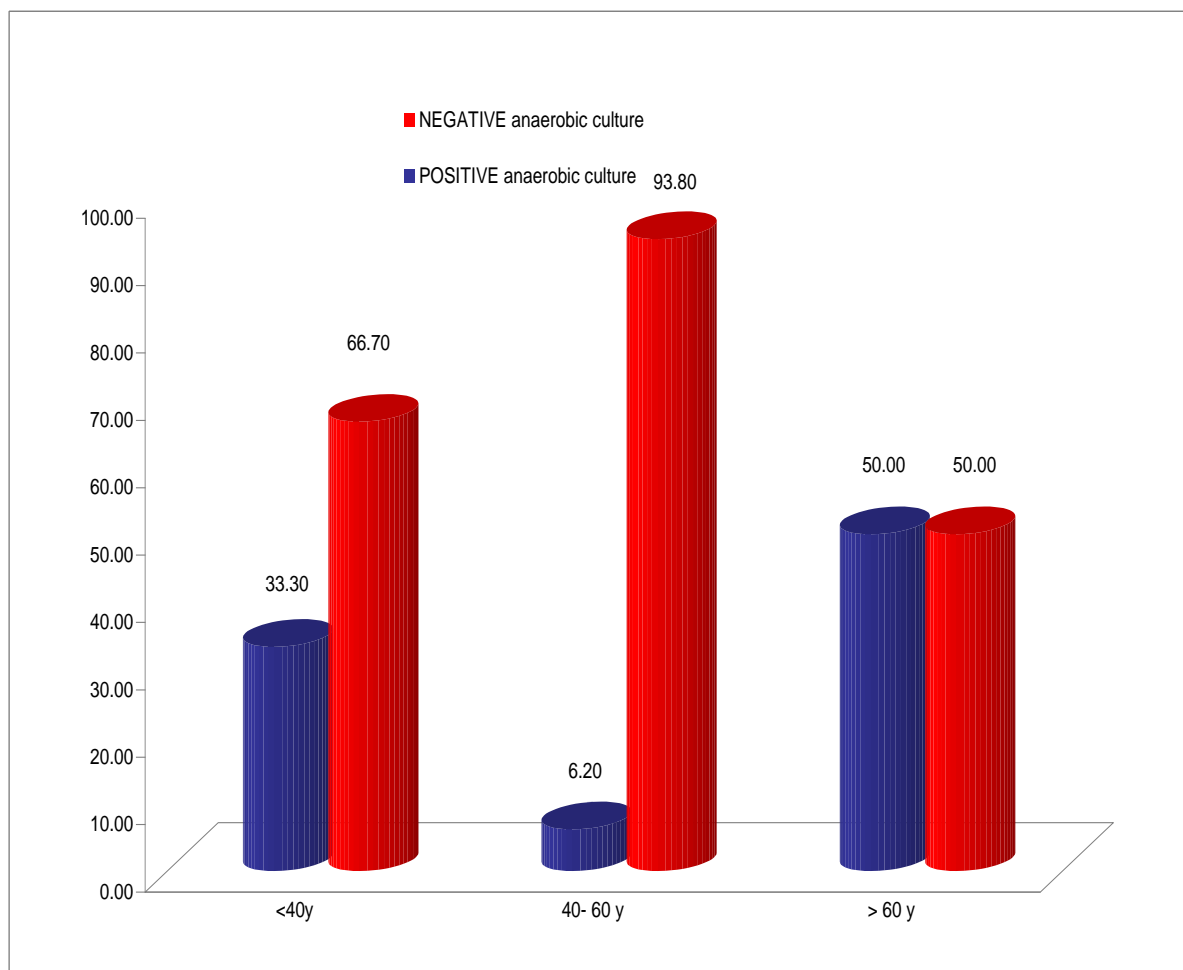
Table (29): Comparison between results of anaerobic culture of tracheal aspirate samples after 72h of intubation according to age group

		<40y		40- 60 y		> 60 y		Total		X^2	P*
		No of cases	%	No of cases	%	No of cases	%	No of cases	%		
After 72h	NEGATIVE CULTURE	2	66.7%	1	6.2%	0	0.0%	3	8.6%	22.1	<0.05
	POSITIVE CULTURE	1	33.3%	15	93.8%	16	100.0%	32	91.4%		
	Total	3	100.0%	16	100.0%	16	100.0%	35	100.0%		

P* :Significant.

Table (27) shows that one (33.3%) out of 3patients under 40 years old ,15 (93.8%)out of 16 patients from 40-60 years old and all patients above 60 years old gave positive anaerobic culture from samples taken after 72 hour of intubation. A difference which is significant.

Figure(21): Comparison between results of anaerobic culture of tracheal aspirate samples after 72h of intubation according to age group
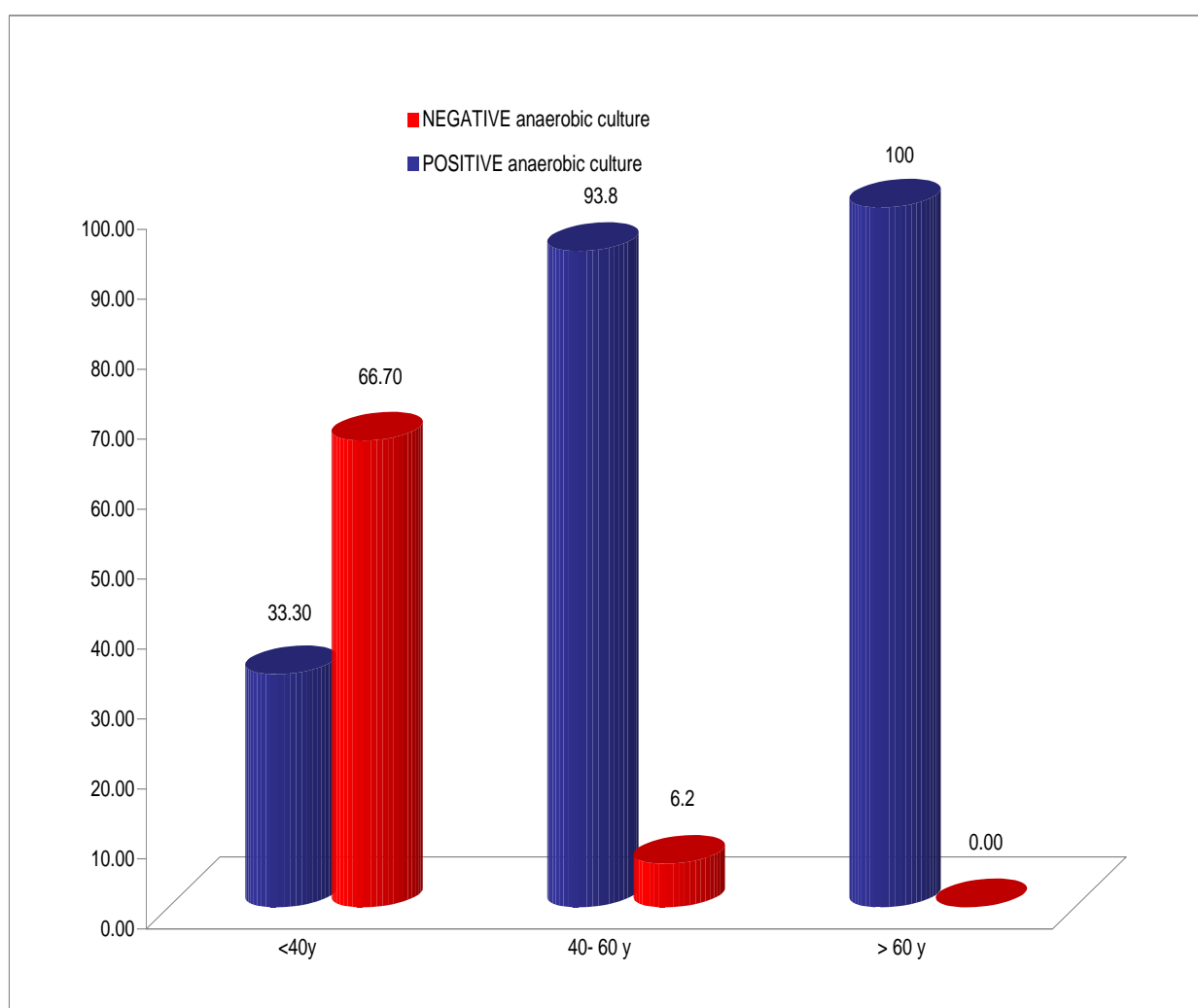
Table (30): Comparison between results of anaerobic culture of tracheal aspirate samples within 24h of intubation as regard sex

		Sex						X^2	p*
		female		Male		Total			
		No of cases	%	No of cases	%	No of cases	%		
within 24h	NEGATIVE CULTURE	12	57.1%	13	92.9%	25	71.4%	5.3	<0.05
	POSITIVE CULTURE	9	42.9%	1	7.1%	10	28.6%		
	Total	21	100.0%	14	100.0%	35	100.0%		

P* :Significant.

Table (28) shows that 9 (42.9%) out of 21 female patients and one (7.1%) out of 14 male patients gave positive anaerobic culture from samples taken within 24 hour of intubation. A difference which is significant

Figure(22): Comparison between results of anaerobic culture of tracheal aspirate samples within 24h of intubation as regard sex
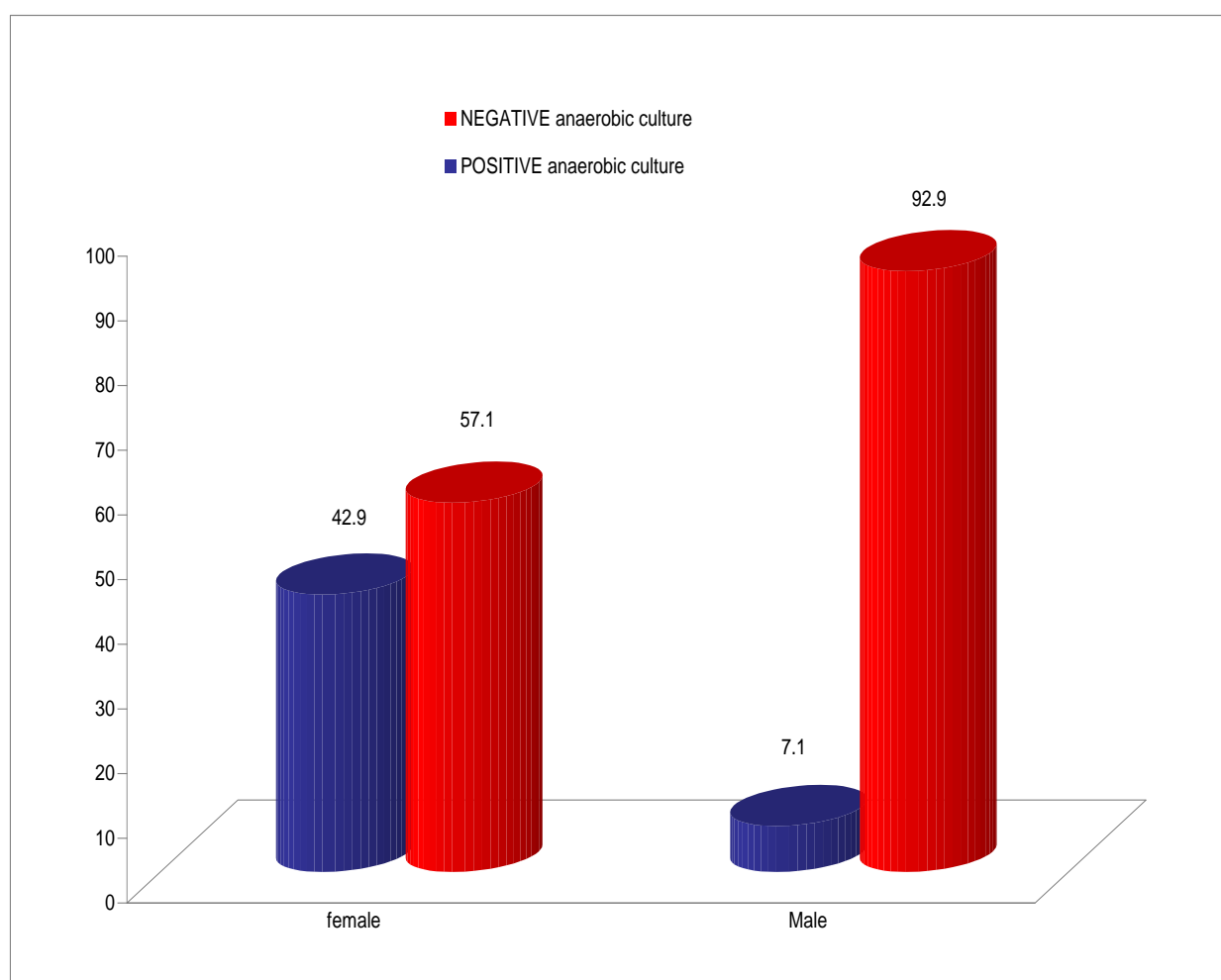
Table (31): Comparison between results of anaerobic culture of tracheal aspirate samples after 72h of intubation as regard sex

		Sex						X^2	P*
		female		Male		Total			
		No of cases	%	No of cases	%	No of cases	%		
After 72h	NEGATIVE CULTURE	3	14.3%	0	.0%	3	8.6%	2.2	>0.05
	POSITIVE CULTURE	18	85.7%	14	100.0%	32	91.4%		
	Total	21	100%	14	100%	35	100.0%		

p*: Insignificant.

Table (29) shows that 18 (85.7%) out of 21 female patients and all male patients gave positive anaerobic culture from samples taken after 72 hour of intubation. A difference which is insignificant

Figure(23): Comparison between results of anaerobic culture of tracheal aspirate samples after 72h of intubation as regard sex
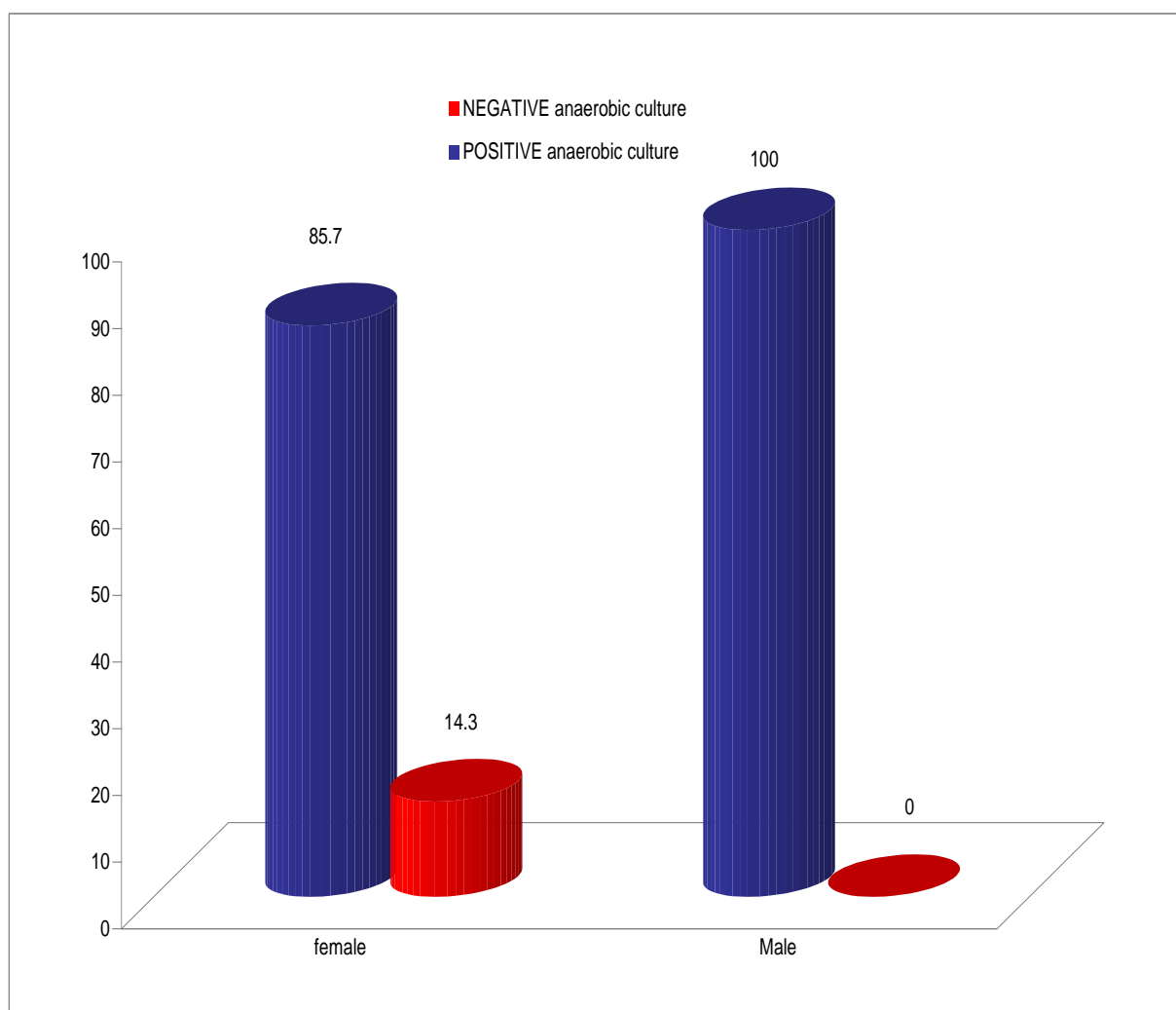
Table (32): Antimicrobial sensitivity pattern of isolated anaerobic organisms in the study by disc diffusion method

	MET	CLIN	CHLOR	CIP	AZITH	CEPO
Lactobacilli	S	S	S	R	S	R
Peptostrept	S	R	I	R	S	R
Prevotella	S	S	S	R	S	R
Fusobacterium	S	R	I	R	I	R
Veillonella	S	S	S	R	S	R
Clostridium	S	R	S	R	I	R
Bacteroides	S	S	S	R	S	R

Table (30) shows that all isolated anaerobic strains were sensitive to metronidazole and resistant to ciprofloxacin and ceporex.

S: sensitive, R: resistant , I: intermediate
MET: metronidazole.
CLIN: clindamycin .
CHOR: chloramaphenicol.
CIP: ciprofloxacin.
AZITH:azithromycin .
CEPO: ceporex.