V - RESULTS

This study started in July 2011 and completed in May 2012, the study was conducted on 70 patients suffering from hemorrhagic diarrhea, out of 70 patients 36 (51.4%) were males and 34 (48.6%) were females, 44 (62.9%) patients lived in rural area while 26 (37.1 %) lived in urban area, the age of all patients ranged between 2 to 60 years with Mean ± SD equal (17.7 ± 15.8) .This is shown in table (1), fig (1) and fig (2).

Table (1) Description of patients with hemorrhagic diarrhea

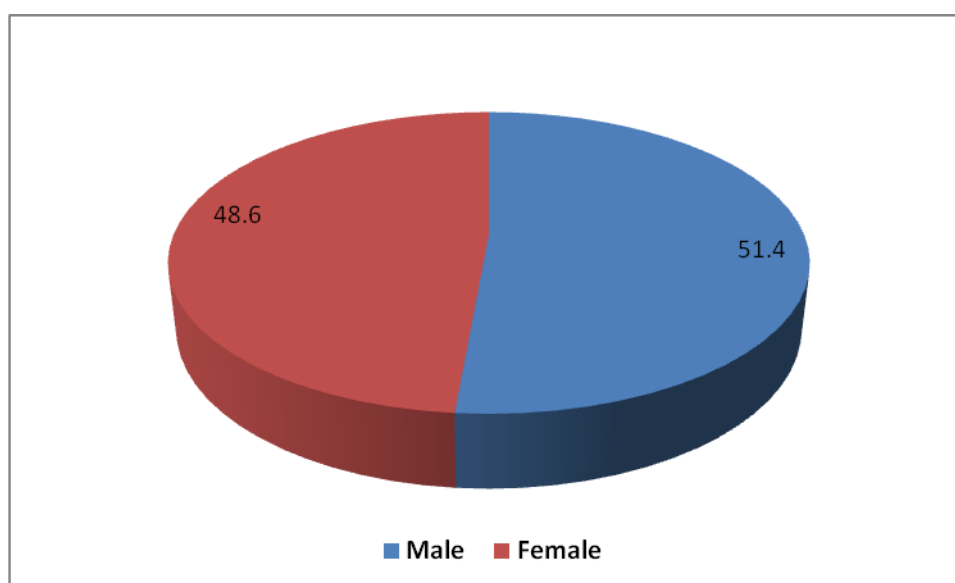| Variable		No. (N=70)	% (100.0)
Gender	Male	36	51.4
	Female	34	48.6
Residence	urban	26	37.1
	rural	44	62.9
Age (years)	Mean ± SD	Min.	Max.
	17.7 ± 15.8	2	60

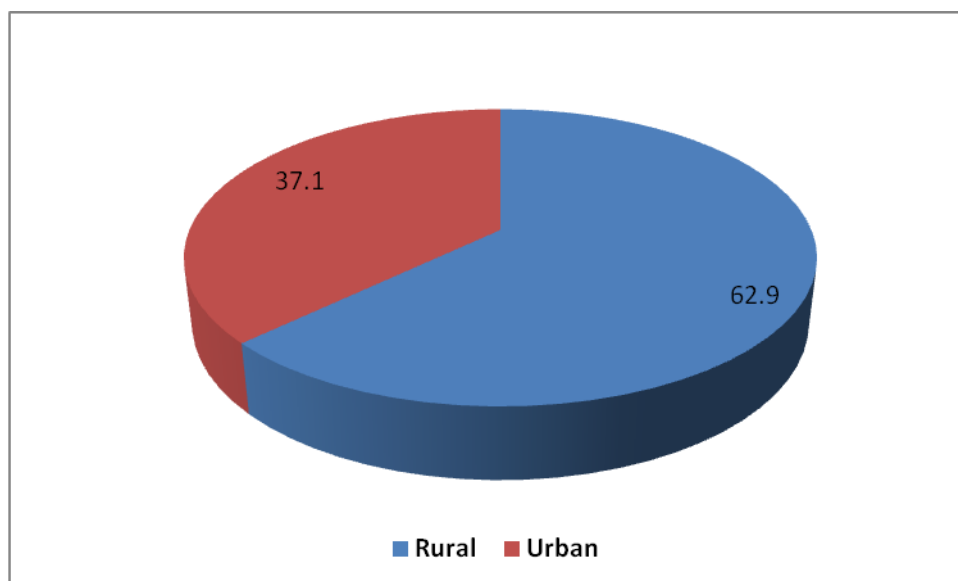**Fig 1: Percentage of gender among patients with hemorrhagic diarrhea.**

Fig 2: Patients with hemorrhagic diarrhea according to residence.

Concerning the clinical presentation of patients with hemorrhagic diarrhea.(vomiting, fever and abdominal pain): vomiting was present in 21.4% of cases, fever was present in 34.3% of cases, while abdominal pain was present in 88.6% of cases, duration of diarrheal episode in days ranges between 1 to 9 days, with Mean ± SD (4.3 ± 1.7) . This is shown in table (2) and fig (3).

Table 2 :Clinical presentation of patients with hemorrhagic diarrhea.

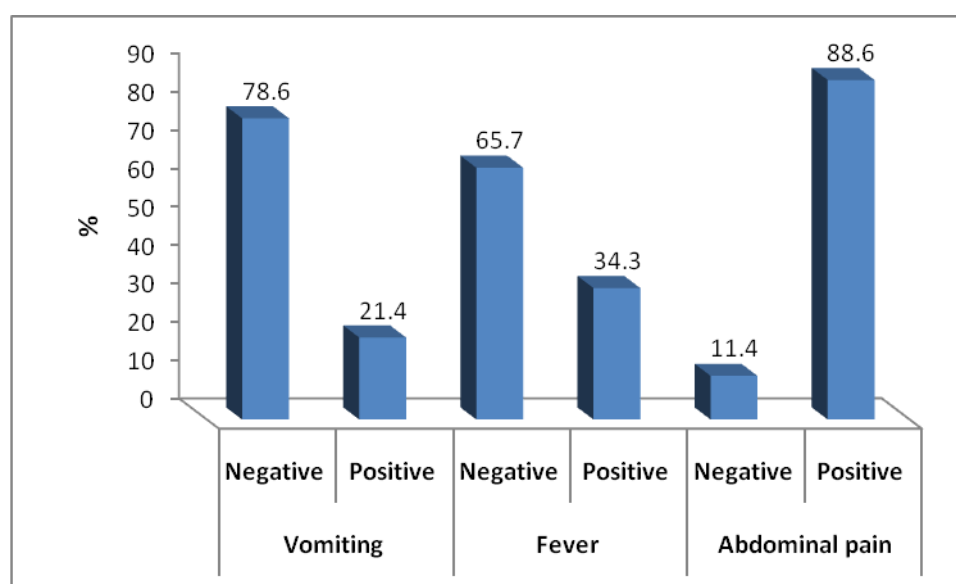| Variable		No. (N=70)	% (100.0)
Vomiting	Negative	55	78.6
	Positive	15	21.4
Fever	Negative	46	65.7
	Positive	24	34.3
Abdominal pain	Negative	8	11.4
	Positive	62	88.6
Diarrheal episode duration (days)	Mean ± SD	Min.	Max.
	4.3±1.7	1	9

Fig 3: Clinical presentation of the patients with hemorrhagic diarrhea.

E. coli was detected in 33 specimens representing (47.1%) of cases and *Shigella* was detected in 28 specimens (40%). while other bacteria can be isolated from 9 specimens (12.9%) .This is shown in table (3) and fig (4) .

Table 3: Organisms isolated from stool samples of patients with hemorrhagic diarrhea.

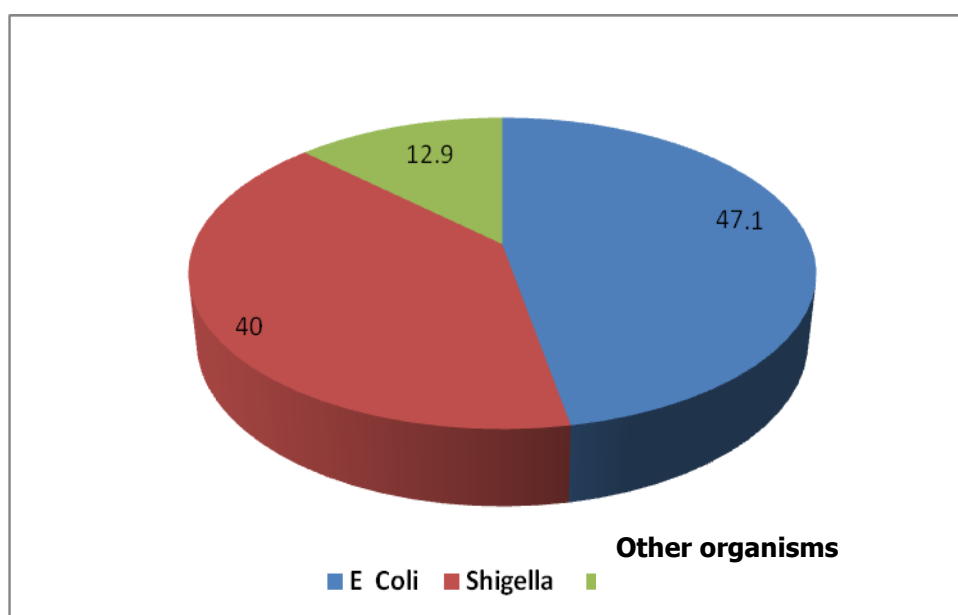| Organism	No. (N=70)	% (100.0)
E. coli	33	47.1
Shigella	28	40.0
Other bacteria	9	12.9

Fig 4: Percentage of isolated organisms from stool samples of patients with hemorrhagic diarrhea.

Out of 33 *E. coli* isolates, 7 (21.2%) isolates were non sorbitol fermenters on sorbitol MacConkey agar while 26 (78.8%) isolates were sorbitol fermenters . This was statistical significant $P < 0.05$ as shown in table (4) and fig (5) .

Table 4 : Effect of isolated *E. coli* on Sorbitol

E. coli	No. (N=33)	% (100.0)	Z test	P
NSF	7	21.2	4.05	<0.001*
SF	26	78.8		

NSF : non sorbitol fermenting
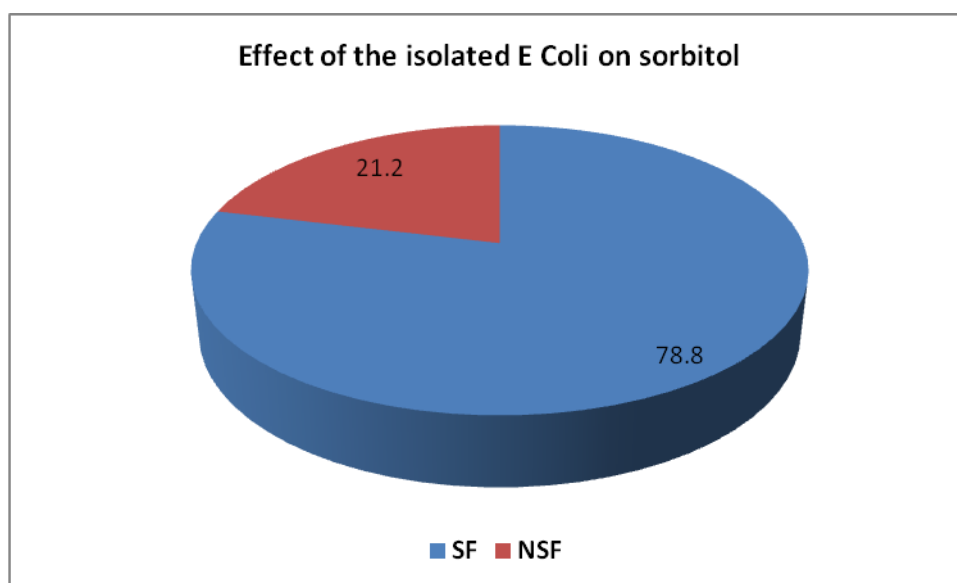SF: sorbitol fermenting

Fig 5: Effect of *E. coli* on sobitol

Fig 6: Effect of non sorbitol fermenting *E. coli* **on sorbitol MacConkey's agar** (*E. coli* **O157**)
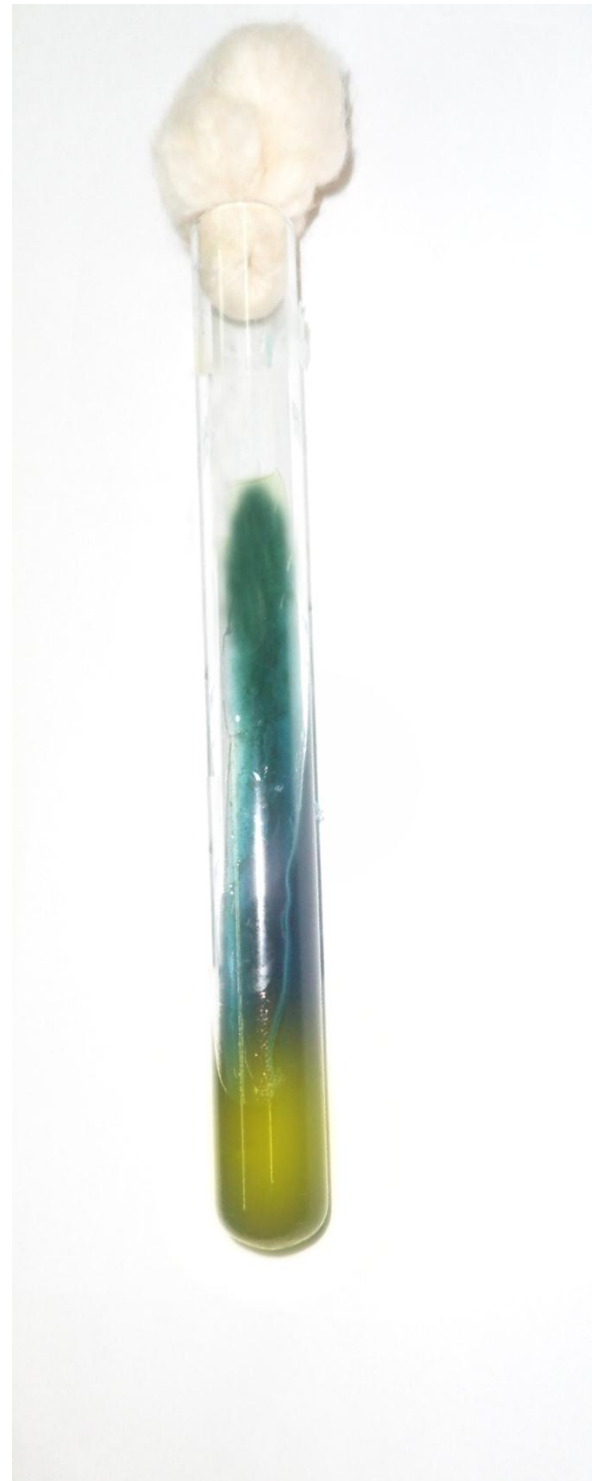
Fig 7 : Effect of sorbitol fermenting *E. coli* **on sorbitol MacConkey's agar**

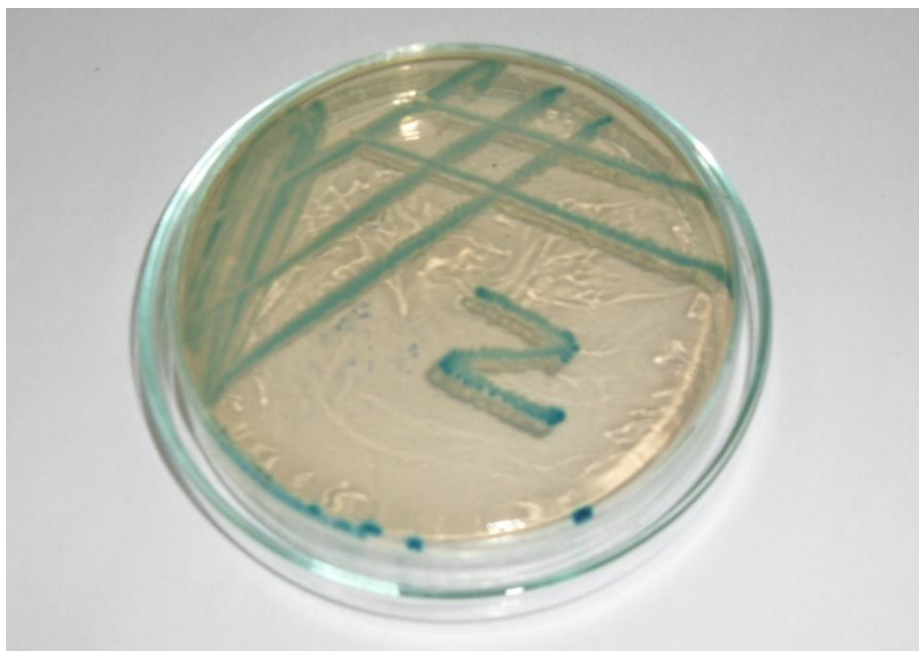On ChromIDTM O157:H7 all NSF *E. coli* (7 cases) gave typical colonies of *E. coli* O157 representing (100%) as shown in table (5) .

Table 5 :Effect of isolated non sorbitol fermenting (NSF) *E. coli* on ChromIDTM O157:H7 agar

NSF *E. coli*	No. (N=7)	% (100.0)
+ ve for *E coli* O 157 by ChromIDTM 0157:H7 agar	7	100
- ve for *E coli* O 157 by ChromIDTM 0157:H7 agar	0	0

Fig 8: ChromIDTM O157:H7 agar

Fig 9: ChromIDTM 0157:H7 show bluish-green color colonies (typical colonies for *E. coli* O 157)

By latex agglutination test : 4 (15.4%) strains of SF *E.coli* were positive for O26 and 6 (23.1%) strains were positive for O111, while 16 (61.5%) strains were negative for O26 and O 111. This is shown in table (6) and fig (10).

Table 6: Results of serotypes of sorbitol fermenting (SF) *E.coli* by latex agglutination test

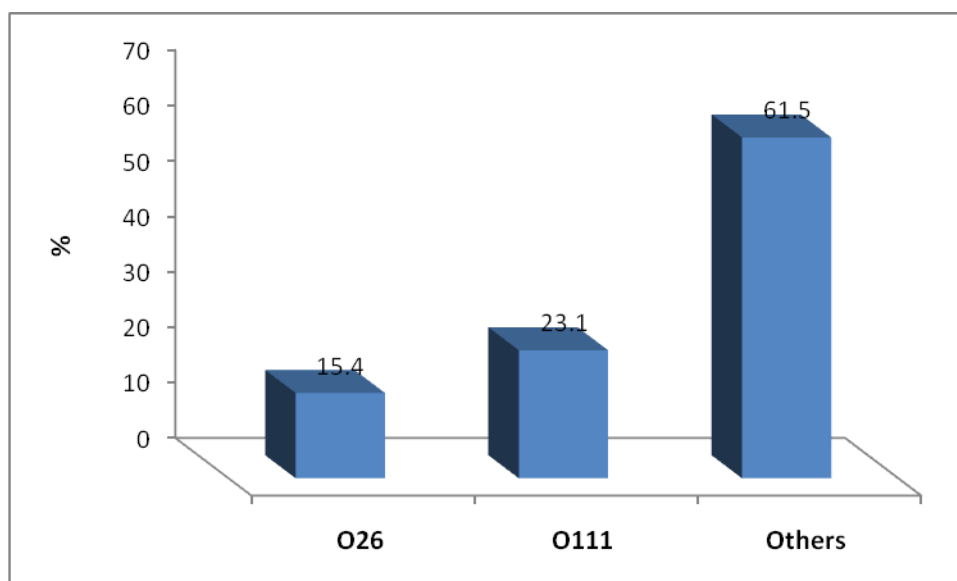| SF *E. coli*	No. (N=26)	% (100.0)
O26	4	15.4
O111	6	23.1
Others	16	61.5

Fig 10: Results of serotypes of SF *E. coli* by latex agglutination test
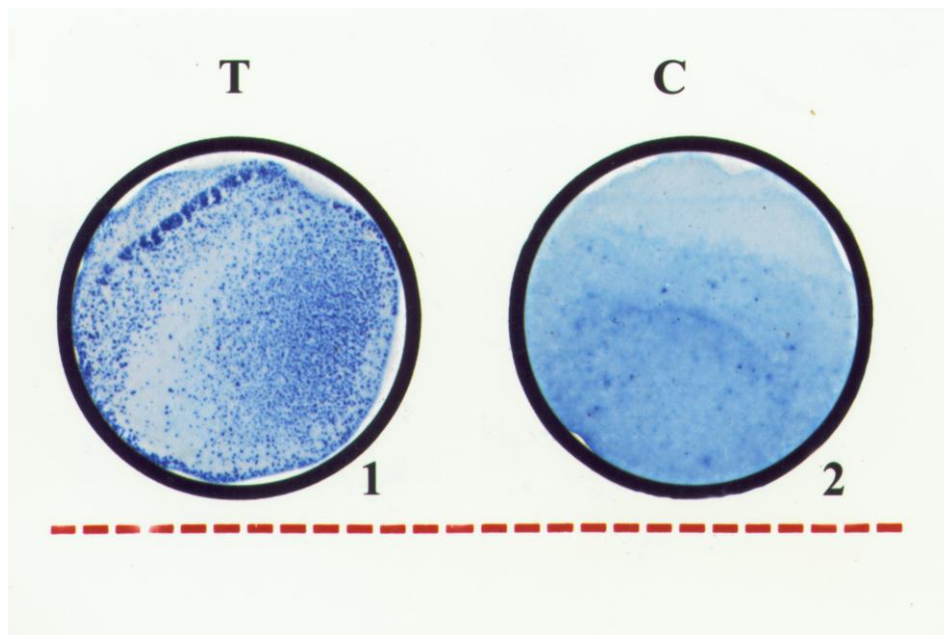
Fig. 11: *E. coli* O111 latex test showing a positive reaction

Circle T: Test circle showing obvious agglutination of the tested strain with the latex reagent.

Circle C: Control circle showing no agglutination of the tested strain with the control latex reagent.

Out of 28 strains of *Shigella* isolated, 7 (25%) strains were non mannitol fermenters while 21 (75%) strains were mannitol fermenters. This was statistical significant $P < 0.05$ as shown in table (7) and fig (12).

Table 7: Effect of isolated *Shigella* on mannitol

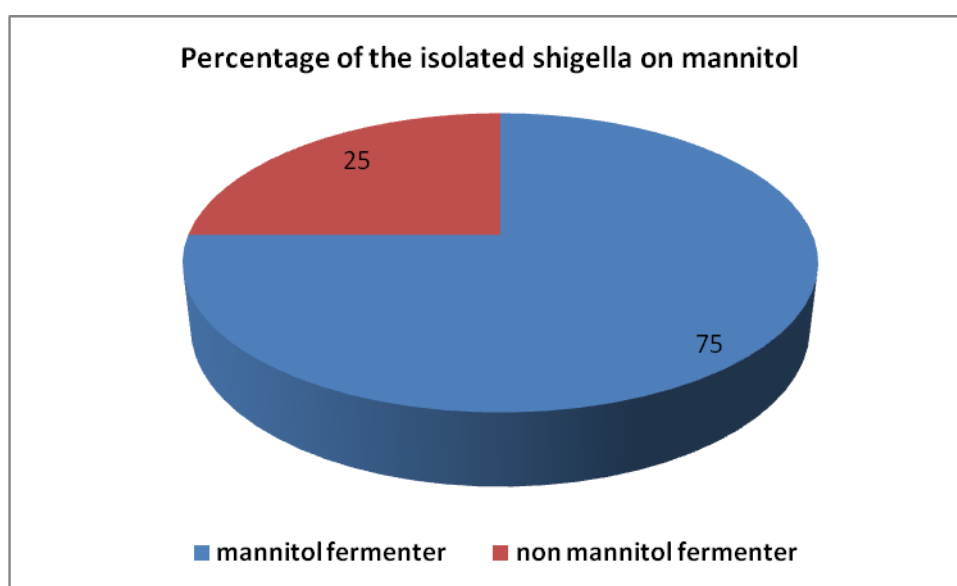| *Shigella*	No. (N=28)	% (100.0)	Z test	P
non mannitol fermenter (*Shigella shiga*)	7	25.0	2.38	0.017*
mannitol fermenter (other *Shigella*)	21	75.0		

Fig 12: Percentage of isolated *Shigella* on mannitol

As regard the organisms isolated from the stool samples: *E. coli* O111 were detected in 6 specimens representing 8.6% of cases, *E. coli* O26 were detected in 4 specimens representing 5.6% of cases, *E. coli* O157 were detected in 7 specimens representing 10% of cases, other *E. coli* were detected in 16 specimens representing 22.9% of cases, *Shigella shiga* was detected in 7 specimens representing 10%, other types of *Shigella* were detected in 21 specimens representing 30% of cases, while other than organisms can be isolated from 9 specimens representing 12.9% of cases. This is shown in table (8).

Table 8 : Types of the organisms isolated from stool of patients with hemorrhagic diarrhea

Types of the organisms	Frequency	Percent
O 111	6	8.6
O 26	4	5.7
O 157	7	10.0
other *E. coli*	16	22.9
Shigella shiga	7	10.0
other *Shigella*	21	30.0
other organisms	9	12.9
Total	70	100.0

As regard of relation between gender and isolated organisms in patients with hemorrhagic diarrhea: 17 (47.2%) *E.coli* were detected among males and 16 (47.1%) were detected among females, 17 (47.2%) *Shigella* were detected among males and 11 (32.4%) were detected among females. Other organisms were detected among 7 (20.6%) males and 2 (5.6%) females. This is shown in table (9).

Table 9 : Relation between gender and isolated organisms in patients with hemorrhagic diarrhea .

			Gender		Total	Z	P
			male	female			
Isolated organism	Other organims	Count	7	2	9	1.88	0.06
		% within gender	20.6%	5.6%	12.9%		
	E. coli	Count	17	16	33	0.014	0.98
		% within gender	47.2%	47.1%	47.1%		
	Shigella	Count	17	11	28	1.27	0.204
		% within gender	47.2%	32.4%	40.0%		
Total		Count	36	34	70	-------	-------
		% within gender	100.0%	100.0%	100.0%		

The mean age of patients isolated *E.coli* was 18.5 years ± 14.9 SD, and for patients isolated *Shigella* was 11.7 years ± 10.3 SD, while the mean age of patients isolated other organisms was 33.3 years ± 22.6 SD . This was statistical significant P < 0.05. This is shown in table (10) and fig (13).

Table 10: Relation between the mean age of patients with hemorrhagic diarrhea and isolated organisms

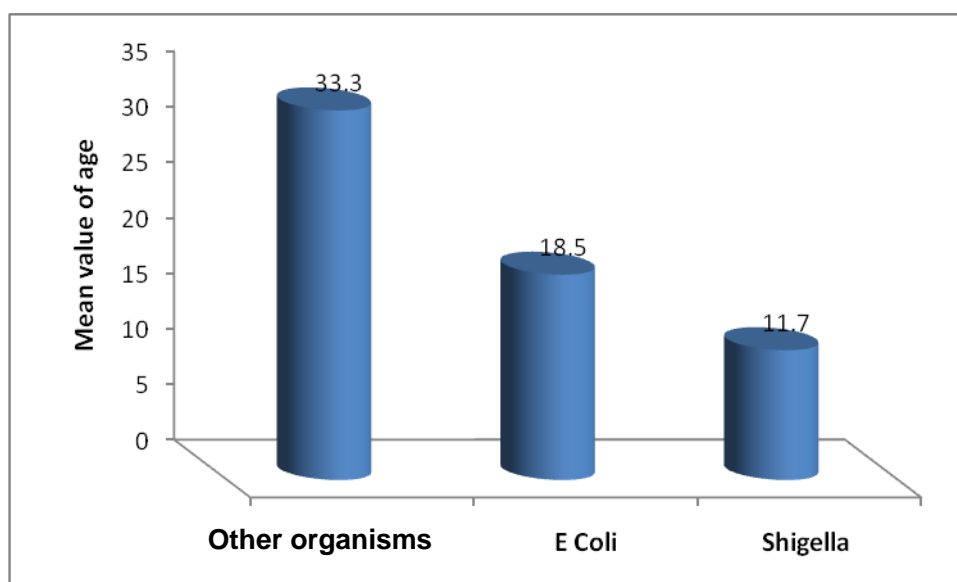| ORGANISMS	Age/year		ANOVA	P
	N	Mean ± SD		
Other organisms	9	33.3 ± 22.6	7.6	0.001*
E coli	33	18.5 ± 14.9		
Shigella	28	11.7 ± 10.3		

Fig 13: Relation between the mean age of patients with hemorrhagic diarrhea and isolated organisms

The mean duration of diarrheal episodes in patients with *E.coli* was 4.2 ± 1.76 SD, the mean Duration of diarrheal episodes in patients with *Shigella* was 4.4 ± 1.64 SD, while the mean duration of diarrheal episodes in patients with other organisms was 4.6 ± 2.06 SD . This is shown in table (11)

Table 11: Relation between the mean duration of diarrheal episode and types of isolated organisms among patients with hemorrhagic diarrhea.

Organism	Duration of diarrheal episodes		ANOVA	P
	N	Mean ± SD		
Other organims	9	4.6 ± 2.06		
E coli	33	4.2 ± 1.76	0.15	0.86
Shigella	28	4.4 ± 1.64		

Vomiting was present in 10 patients with *E. coli* and present in 5 patients with *Shigella* but not present in any patient with other organisms, so that the vomiting was present in 15 cases representing (34.2%). This is shown in table (12)

Table 12: Relation between vomiting and isolated organisms in patients with hemorrhagic diarrhea .

Isolated organism		vomiting		Total No	Z	P
		negative	positive			
Other organims	Count	9	0	9	1.68	0.092
	%	16.4%	.0%	12.9%		
E. coli	Count	23	10	33	1.71	0.08
	%	41.8%	66.7%	47.1%		
Shigella	Count	23	5	28	0.6	0.54
	%	41.8%	33.3%	40.0%		
Total	Count	55	15	70	---------	-----------
	%	100.0%	100.0%	100.0%		

As regard of relation between fever and isolated organisms in patients with hemorrhagic diarrhea: Fever was present in 9 patients with *E.coli* and present in 14 patients with *Shigella* while it was present in 1 patient with other organisms, so that the fever was present in 24 case representing (34.4%). There was statistical significant difference in frequency distribution of patients with hemorrhagic diarrhea according to fever with *Shigella* but not significant with *E. coli* and other organisms . This is shown in table (13) and fig (14).

Table 13: Relation between fever and isolated organisms in patients with hemorrhagic diarrhea .

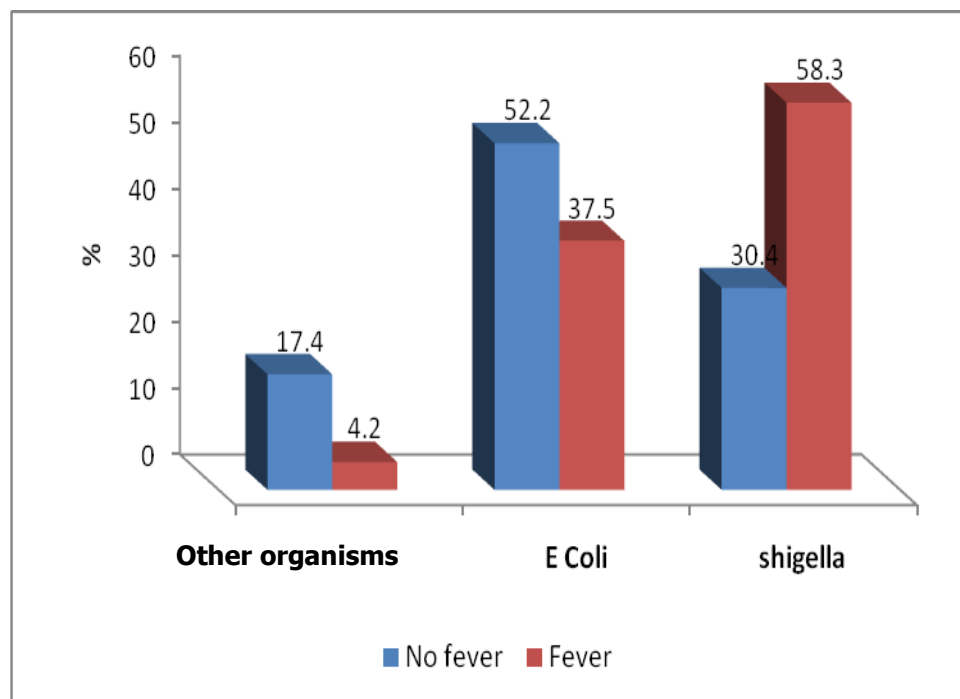| Isolated organism		Fever		Total No	Z	P
		negative	positive			
Other organisms	Count	8	1	9	1.6	0.1
	%	17.4%	4.2%	12.9%		
E. coli	Count	24	9	33	1.17	0.24
	%	52.2%	37.5%	47.1%		
Shigella	Count	14	14	28	2.26	0.02*
	%	30.4%	58.3%	40.0%		
Total	Count	46	24	70	---------	-----------
	%	100.0%	100.0%	100.0%		

Fig 14: Relation between fever and isolated organisms in patients with hemorrhagic diarrhea .

As regards the relation between abdominal pain and isolated organisms in patients with hemorrhagic diarrhea: abdominal pain was present in 28 patients with *E. coli* and present in 28 patients with *Shigella* while it was present in 6 patients with other organisms, so that the abdominal pain was present in 62 cases representing (88.5%) . There was statistical significant difference in frequency distribution of the studied samples according to abdominal pain with *Shigella* and other organisms but not significant with *E. coli.* This is shown in table (14) and fig (15).

Table 14: Relation between abdominal pain and isolated organisms in patients with hemorrhagic diarrhea .

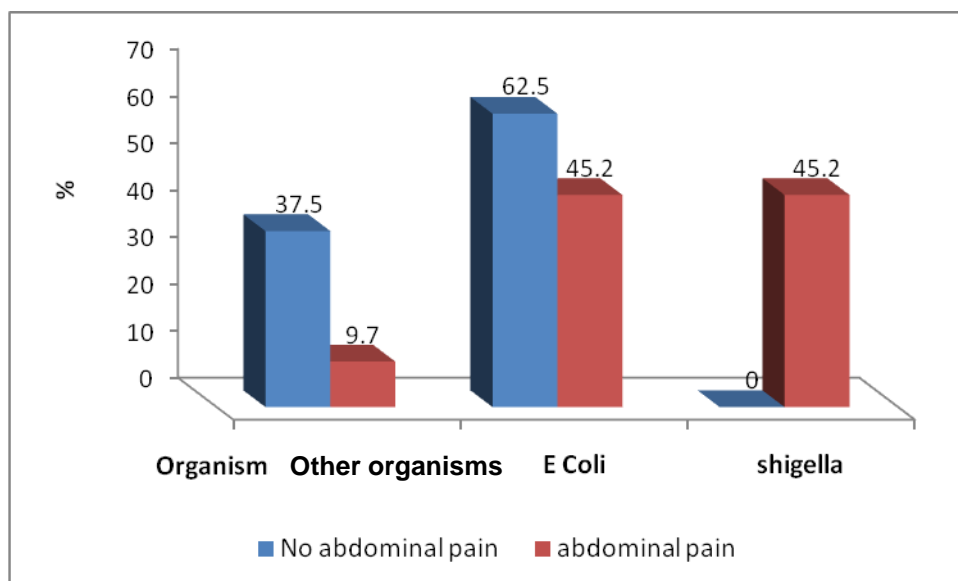| Isolated organism		Abdominal pain		Total	Z	P
		negative	positive			
Other organisms	Count	3	6	9	2.21	0.026*
	%	37.5%	9.7%	12.9%		
E. coli	Count	5	28	33	0.93	0.34
	%	62.5%	45.2%	47.1%		
Shigella	Count	0	28	28	2.45	0.014*
	%	.0%	45.2%	40.0%		
Total	Count	8	62	70	---------	-----------
	%	100.0%	100.0%	100.0%		

Fig 15: Relation between abdominal pain and isolated organisms in patients with hemorrhagic diarrhea .

By comparing *E. coli* 157 and *Shigella shiga* infection as regard age it was found that : The mean age of patients with *E. coli* O 157 was 24 years ± 14.5 Sd, while the mean age of patients with *Shigella shiga* was 7.8 years ± 1.9 SD . There was statistical significant difference (P < 0.05) . This is shown in table (15) and fig (17).

Table 15 :Comparing *E. Coli* 157 and *Shigella shiga* infection regarding age of patients.

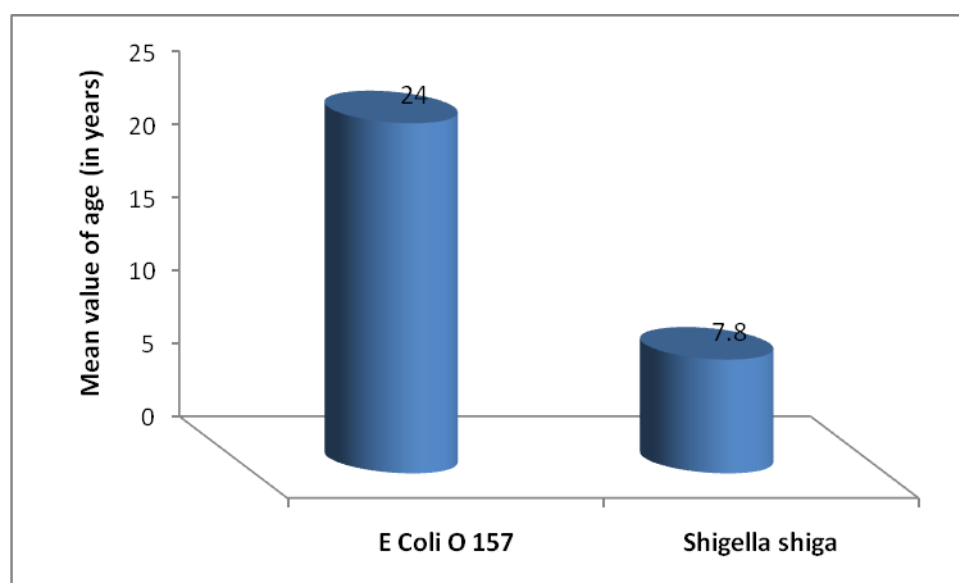| ORGANISMS	Age/year		St. "t" test	P
	N	Mean ± SD		
O 157	7	24.0 ± 14.5	2.9	0.013*
Shigella shiga	7	7.8 ± 1.9		

Fig 16 : Mean value of age (in years)

By comparing *E. coli* 157 and *Shigella shiga* infection as regard duration of diarrheal episode it was found that: The mean duration of diarrheal episode of patients with *E. coli* O 157 was 4.6 ± 1.5 SD, while the mean duration of diarrheal episode of patients with *shigella shiga* was 3.7 ± 0.76 SD . There was statistical insignificant difference (P < 0.05) . This is shown in table (16) and fig (18).

Table 16 : Comparing *E. coli* 157 and *Shigella shiga* infection regarding duration of diarrheal episode.

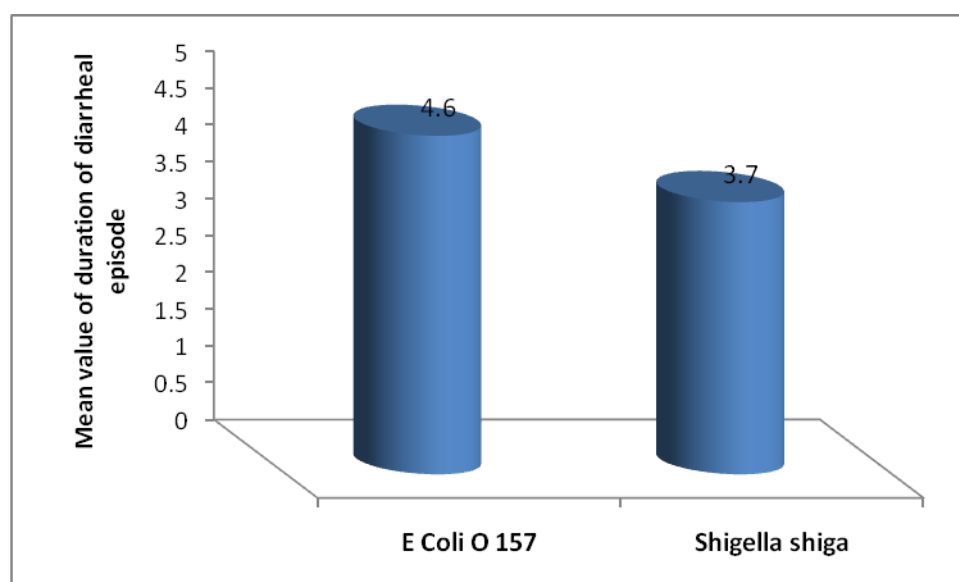| ORGANISMS	Duration of diarrheal episode		St. "t" test	P
	N	Mean ± SD		
O 157	7	4.6 ± 1.5	1.34	0.205
Shigella shiga	7	3.7 ± 0.76		

Fig 17: Mean value of duration of diarrheal episode.

Table (17) shown comparison between *E. coli* 157 and *Shigella shiga* infection in patients with hemorrhagic diarrhea as regard residence, gender, vomiting, fever and abdominal pain it was found that :

According to residence: 2 patients with *E. coli* O 157 were living in urban areas (28.6%) while 5 patients were living in rural areas (71.4%) and 7 patients with *Shigella shiga* were living in urban areas (100%) while no patient living in rural areas (28.6%)

According to gender: 5 patients were males (71.4%) and 2 were females (28.6%) in patients with *E. coli* O 157, while 4 patients were males (57.1%) and 3 were females (42.9%) in patients with *Shigella shiga.*

According to vomiting: 14.3% of cases with *E. coli* O 157 were presented with vomiting while 85.7% were not presented with vomiting, 28.6% of cases with *Shigella shiga* were presented with vomiting while 71.4% were not presented with vomiting.

According to fever: 42.9% of cases with *E. coli* O 157 were presented with fever while 57.1% were not presented with fever, 71.4% of cases with *Shigella shiga* were presented with fever while 28.6% were not presented with fever.

According to abdominal pain: 100% of cases with *E. coli* O 157 were presented with abdominal pain and also in cases with *Shigella shiga*.

Table 17 :Comparing *E. coli* 157 and *Shigella shiga* infecion regarding to residence, gender, vomiting, fever and abdominal pain

|			Organism		Total
			O 157	*Shigella shiga*	
residence	urban	Count	2	0	2
		% within organism	28.6%	.0%	14.3%
	rural	Count	5	7	12
		% within organism	71.4%	100.0%	85.7%
gender	male	Count	5	4	9
		% within organism	71.4%	57.1%	64.3%
	female	Count	2	3	5
		% within organism0	28.6%	42.9%	35.7%
vomiting	negative	Count	6	5	11
		% within organism	85.7%	71.4%	78.6%
	positive	Count	1	2	3
		% within organism	14.3%	28.6%	21.4%
fever	negative	Count	4	2	6
		% within organism0	57.1%	28.6%	42.8%
	positive	Count	3	5	8
		% within organism	42.9%	71.4%	57.1%
abdominal pain	positive	Count	7	7	14
		% within organism	100.0%	100.0%	100.0%
	Negative	Count	0	0	0
		% within organism	0%	0%	0%
Total		Count	7	7	14
		% within organism	100.0%	100.0%	100.0%
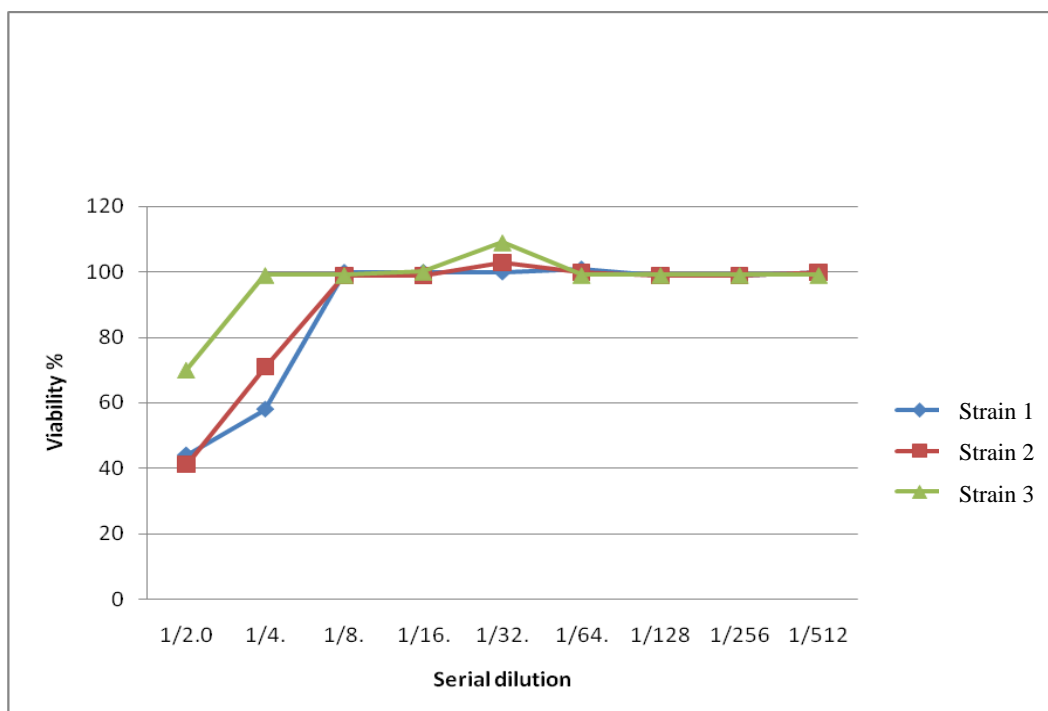
Fig 18: Evaluation of viability % of vero cells treated with *E. coli* O157 toxin using the tetrazolium-based colorimetric assay (MTT assay). Strains 1 , 2 and 3
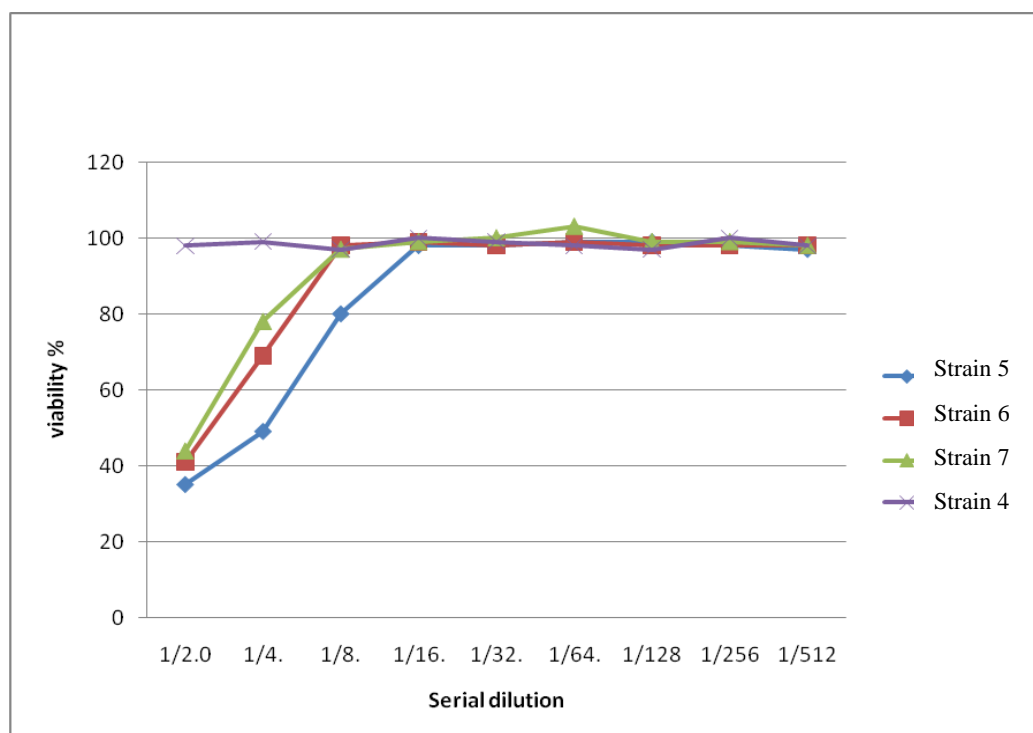

Fig 19: Evaluation of viability % of vero cells treated with *E. coli* O157 toxin using the tetrazolium-based colorimetric assay (MTT assay). Strains 4 , 5 , 6 and 7

Fig 18 and 19 revealed that 6 out of 7 (85.7 %) showed a reasonable toxicity to Vero cells post treatment with Bacterial toxin while strain No 4 showed no cytotoxic effect on treated cells . The sample toxicity to Vero cells were arranged in the order of strain (1 and 7) 44% , strain (5) 35% , strain (2 and 6) 41% , strain (3) 70% and strain (4) 98.4% . This means that strain No (5) showed a higher toxicity followed by strains No (2 and 6) , (1and 7) and 3 . Strain No (4) showed no toxic effect .

Fig 20 :Normal non treated vero cells
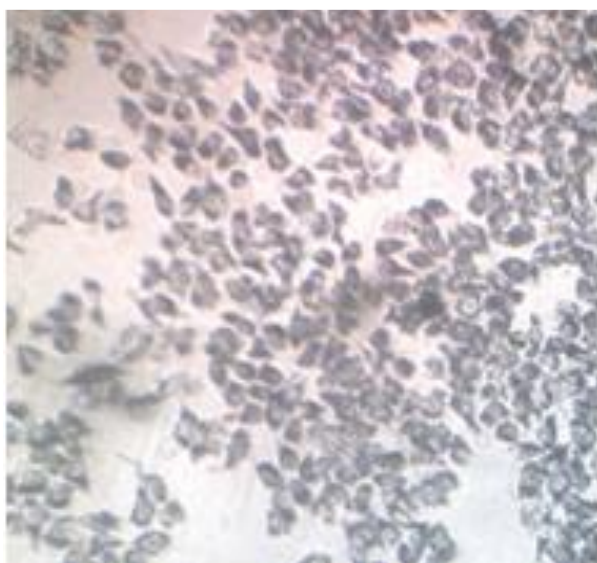
Fig 21 :Vero cell cytoxicty post *E. coli* toxin treatment using the tetrazolium-based colorimetric assay (MTT assay) Stained with MTT