Results

RESULTS

The present study was carried out on 80 subjects divided into 3 groups :

Group I : "Hospitalized diarrheal group".

Included 40 hospitalized patients, received antibiotics and developed diarrhea. Their ages ranged from 18-90 years.

Group II : "Hospitalized non-diarrheal gorup".

Included 20 hospitalized patients, did not suffer from diarrhea. Their ages ranged from 18- 90 years.

Group III : "Healthy control group"

Included 20 normal apparently healthy subjects. Their ages ranged from 18-60 years.

Table (2) : **Epidemiological data of the studied groups**

Variable	Group I (40)		Group II (20)		Group III (20)	
	No.	%	No.	%	No.	%
Sex						
-Male	30	75	11	55	10	50
-Female	10	25	9	45	10	50
Mean age ± SD	51.7± 19.9		40.1 ± 18.2		37.0 ± 11.5	

Table (3) : **Clinical data of the hospitalized patients of group I & group II**

Variable	Group I (40)		Group II (20)	
	No.	%	No.	%
Manifestations :				
- Fever ≃ 38°C.	15	37.5	6	30.0
- Abdominal pain.	10	25.0	3	15.0
Underlying diseases :				
- Malignancies.	15	37.5	6	30
- Liver diseases.	5	12.5	4	20
- Abdominal surgeries	5	12.5	3	15
- Renal diseases.	5	12.5	3	15
- Medical diseases.	5	12.5	2	10
- P.U.O.	5	12.5	2	10
Duration of hospital stay:				
- 3-10 days.	25	62.5	16	80
- >10 days	15	37.5	4	20
Tube feeding	10	25	6	30

Table (4) : **Antibiotic therapy given to the hospitalized patients of group I and group II**

Variable	Group I (40)		Group II (20)	
	No.	%	No.	%
Type of antibiotics :				
- Cephalosporins	20	50	11	55
- Ampicillins.	12	30	6	30
- Clindamycins.	4	10	1	5
- Macrolides	4	10	3	15
Rout of administrations :				
- Oral.	5	12.5	5	25
- I.V.	28	70	15	75
- I.M.	7	17.5	0	0
Duration of administrations :				
- 3-10 days.	25	62.5	12	60
- >10 days	15	37.5	8	40

Table (5) : **Rate of isolation of *C.difficile* as detected by anaerobic culture among the studied groups.**

Group	Group I (40)		Group II (20)		Group III (20)		P.value
	No.	%	No.	%	No.	%	
Isolation	2	5	0	0	0	0	0.35 (NS)

This table shows that :

- In group I : Isolation rate was 5%.
- In group II & III : All stool specimens were negative for culture.
- P. value = 0.35 (Non-significant) (NS).

Table (6) : **Incidence rate of toxigenic *C.difficile* as detected by EIA and cell culture assay (C.C.A) among the studied groups**

Group	Group I (40)		Group II (20)		Group III (20)		P.value
	No.	%	No.	%	No.	%	
- Total toxigenic.	4	10	0	0	0	0	0.12 (NS)
- +ve by EIA alone.	4	10	0	0	0	0	
- +ve by C.C.A. alone.	4	10	0	0	0	0	
- + ve by both tests.	4	10	0	0	0	0	

This table shows that :

- In group I : Incidence rate of toxigenic *C.difficile* was 10%.
- All cases +ve by EIA were also +ve by C.C.A.
- In group II & III : All stool specimens were negative for toxin assay.
- P.value = 0.12 (Non-signification) (NS).

Table (7) : **Results of the three tests used for detection of *C.difficile.***

Variable	Total toxigenic (4)	
	No.	%
+ ve by anaerobic culture.	2	50
+ ve by both EIA & C.C.A	4	100
+ ve by the three tests.	2	50

This table shows that :

- Out of the 4 total toxigenic *C.difficile* cases detected by EIA & C.C.A, only 2 cases were also positive by culture, however, the other 2 cases were negative.

Fig. (3) : Results of the three tests used for detection of C.difficile

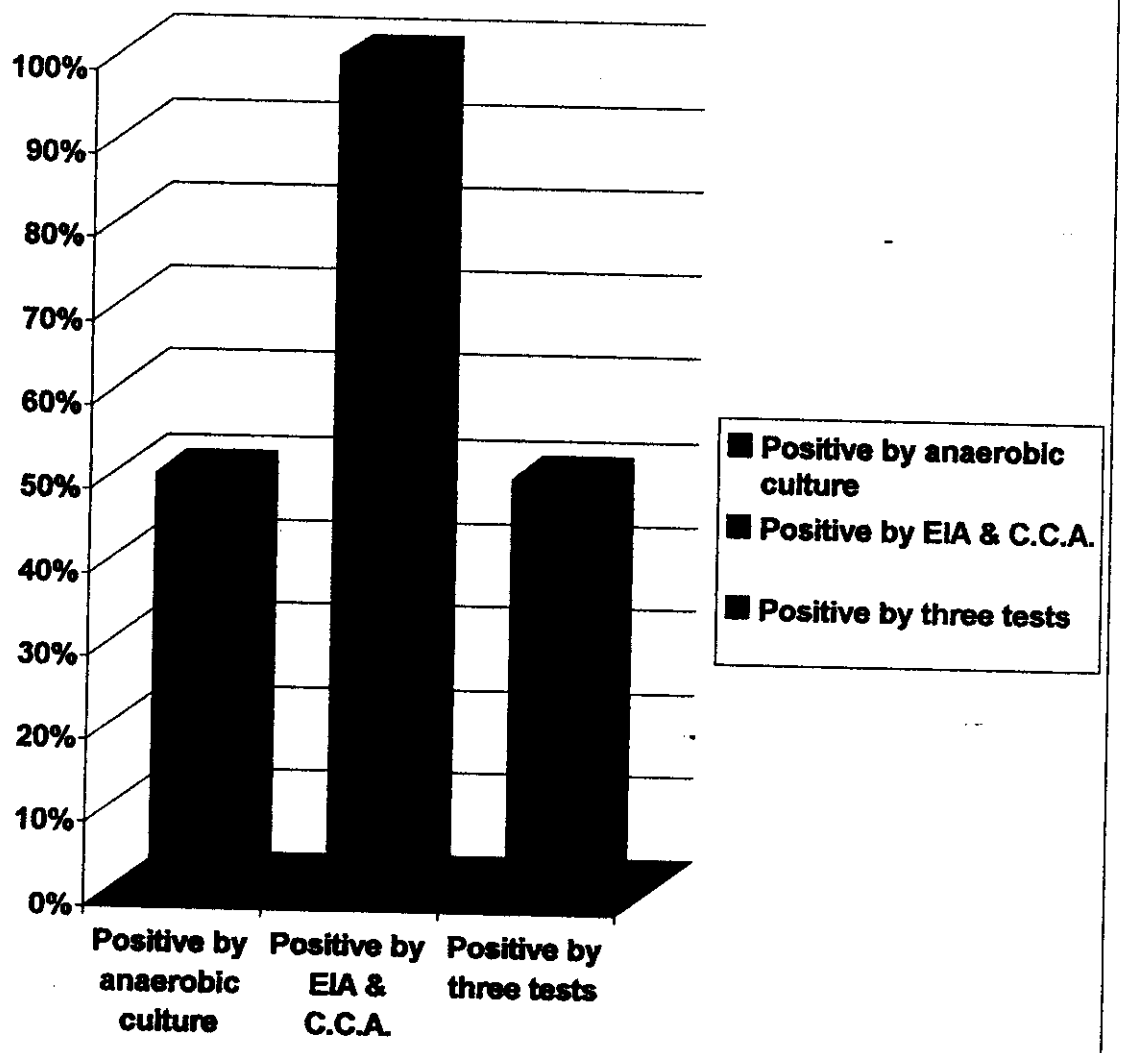

Table (8) : **The incidence rate of toxigenic *C.difficile* in the hospitalized diarrheal patients (group I) according to epidemiological parameters.**

Variable	Total No.. (40)	+ve cases (4)		-ve cases (36)		P.value
		No..	%	No..	%	
Sex						
- Male	30	1	25	29	80.6	0.04 (S)*
- Female	10	3	75	7	19.4	
Mean age ± SD	51.7 ± 19.9	58.2 ± 15.1		39.1 ± 13.9		<0.05 (S)*

This table shows :

- A higher incidence rate of toxigenic *C.difficile* in the stool samples of female patients (75%) than in that of male patients (25%) and this difference is statistically significant. P. value = 0.04 (Significant) (S).
- Mean age of 58.2 ± 15.1 for the +ve cases. P.value < 0.05 (Significant) (S).

Table (9) : **The incidence rate of toxigenic *C.difficile* in the hospitalized diarrheal patients (group I) according to different clinical manifestations.**

Variable	Total No. (40)	+ve cases (4)		-ve cases (36)		P.value
		No.	%	No.	%	
Manifestations :						
- Fever.	15	4	100	11	30.6	0.006 (HS)**
- Abdominal pain.	10	4	100	6	16.7	0.0002 (HS)**

This table shows that :

- Fever is the presenting manifestation in all +ve cases (100%) with p.value = 0.006 (Highly significant) (HS).
- Abdominal pain is the presenting symptom in all +ve cases (100%) with p.value = 0.0002 (Highly significant) (HS).

Table (10) : **Characteristics of diarrhea in +ve and –ve cases of the hospitalized diarrheal patients (group I).**

Variable	Total No. (40)	+ve cases (4)		-ve cases (36)		P.value
		No.	%	No.	%	
Duration :						
- 1-5 days.	30	1	25	29	80.56	0.014 (S)*
- > 5 days.	10	3	75	7	19.44	
Frequency :						
-<10 motions/ day.	35	1	25	34	94.4	0.0001 (HS)**
->10 motions/day.	5	3	75	2	5.6	
Onset in relation to antibiotic therapy :						0.27 (NS)
- 1-10 days.	37	3	75	34	94.4	
- >10 days.	3	1	25	2	5.6	

This table shows :

- A higher incidence rate of toxigenic *C.difficile* in stool samples of hospitalized diarrheal patients suffering from diarrhea > 5 days than patients suffering from diarrhea 1-5 days, and this difference is statistically significant. P. value = 0.014 (S).
- A higher incidence rate of toxigenic *C.difficile* in stool samples of hospitalized diarrheal patients suffering from high frequency of diarrhea >10 motions/day than those suffering from diarrhea 1-10 motions/day and this difference is statistically highly significant P. value = 0.0001 (HS).
- A higher incidence rate of toxigenic *C.difficile* in stool samples of hospitalized diarrheal patients starting diarrhea 1-10 days after antibiotic therapy than patients starting it > 10 days after antibiotic therapy and this difference is statistically insignificant. P. value = 0.27 (NS).

Table (11) ; **The incidence rate of toxigenic *C.difficile* in the hospitalized diarrheal patients (group I) according to the duration of hospital stay.**

Variable	Total No.. (40)	+ve cases (4)		-ve cases (36)		P.value
		No.	%	No.	%	
Duration of hospital stay :						
- 3-10 days.	25	1	25	24	66.7	0.13 (NS)
- >10 days	15	3	75	12	33.3	

This table shows :

- A higher incidence rate of toxigenic *C.difficile* in the stool samples of hospitalized diarrheal patients stayed in the hospital > 10 days (75%) than patients stayed 3-10 days (25%) and this difference is statistically insignificant. P. value = 0.13 (NS).

Fig. (4) : The percentage of toxigenic *C.difficile* in the hospitalized diarrheal patients (group I) according to the duration of hospital stay.

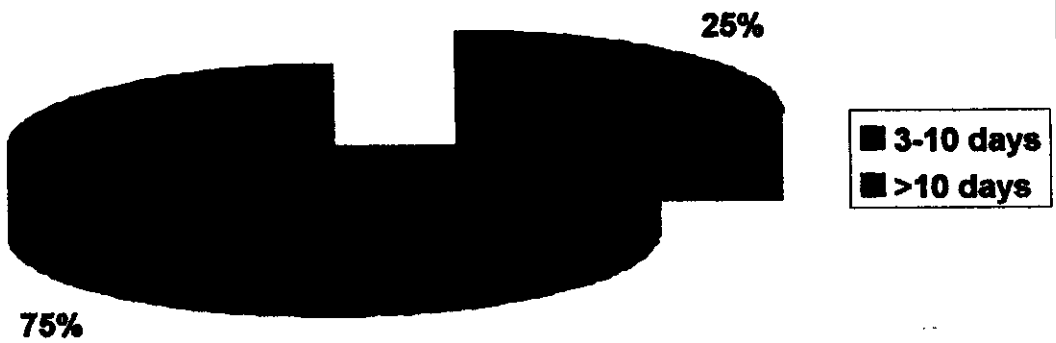

Fig. (5) : The percentage of toxigenic *C.difficile* in the hospitalized diarrheal patients (group I) according to the underlying diseases

Table (13) : **The incidence rate of toxigenic *C.difficile* in the hospitalized diarrheal patients (group I) according to tube feeding.**

Variable	Total No. (40)	+ve cases (4)		-ve cases (36)		P.value
		No.	%	No.	%	
Tube feeding	10	3	75	7	19.4	0.014 (S)*

This table shows :

- Tube feeding was a significant feature in (75%) of +ve cases. P.value = 0.014 (Significant) (S).

Table (14) : **The incidence rate of toxigenic *C.difficile* in the hospitalized diarrheal patients (group I) according to antibiotic therapy**

Variable	Total No. (40)	+ve cases (4)		-ve cases (36)		P.value
		No.	%	No.	%	
Type of antibiotic :						
-Cephalosporins.	20	3	75	17	47.2	0.75 (NS)
-Ampicillins.	12	1	25	11	30.6	0.65 (NS)
-Clindamycins.	4	0	0	4	11.1	0.64 (NS)
-Macrolides.	4	0	0	4	11.1	0.64 (NS)
Rout of administration :						
- Oral.	5	0	0	5	13.9	0.57 (NS)
- I.V.	28	4	100	24	66.7	0.22 (NS)
- I.M.	7	0	0	7	19.9	0.44 (NS)
Duration of administration :						
3-10 days.	25	1	25	24	66.7	0.57 (NS)
>10 days.	15	3	75	13	36.1	0.16 (NS)

This table shows that :

- The hospitalized diarrheal patients receiving cephalosporins had the highest rate of toxigenic *C.difficile* (75%) followed by ampicillin (25%).
- All +ve cases (100%) received antibiotics intravenously.
- A higher incidence rate of toxigenic *C.difficile* in the stool samples of hospitalized diarrheal patients received antibiotics > 10 days (75%) than patients received antibiotics for 3-10 days (25%).

Fig. (6) : The percentage rate of toxigenic C.difficile in the diarrheal patients (group I) according to antibiotic therapy

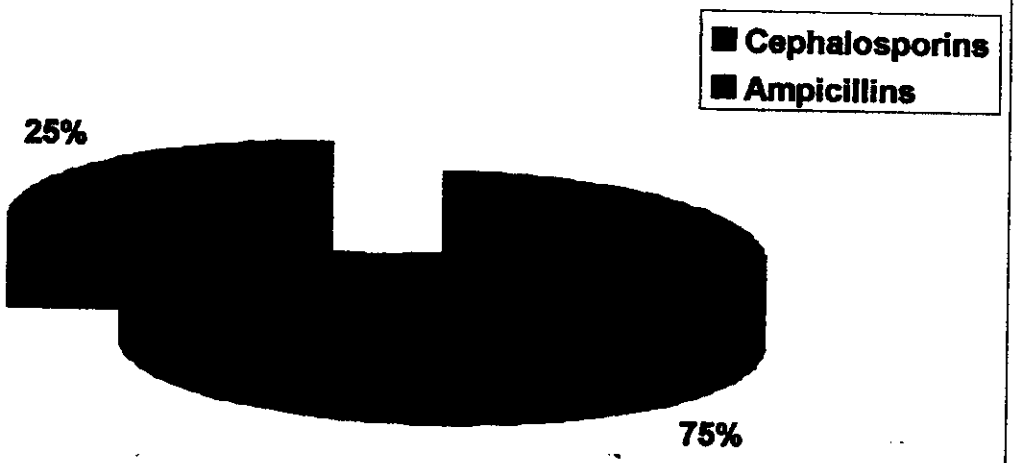

Table (15) : **Data of total +ve cases according to different parameters (total No. 4)**

Data	No. of +ve cases
Epidemiological data :	
- Age (mean age ± SD).	58.2 ± 15.1
- Sex :	
* Male.	1
* Female.	3
Clinical data :	
- Presence of fever and abdominal pain.	4
- Features of diarrhea :	
*Duration of diarrhea.	
<5 days.	1
>5 days.	3
*Frequency of diarrhea.	
<10 motions/ day.	1
>10 motions/day.	3
*Onset of diarrhea in relation to antibiotic.	
1-10days.	3
>10 days.	1
- Underlying disease :	
*Malignancies	2
*Abdominal surgery.	1
*Liver diseases.	1
*Renal diseases.	0
*Medical diseases.	0
*P.U.O. diseases	0
- Tube feeding.	3
- Duration of hospital stay.	
<10 days.	1
>10 days.	3
Antibiotic therapy :	
- Type of antibiotic.	
*Cephalosporins.	3
*Ampicillin.	1
*Clindamycins.	0
*Macrolides.	0
- Rout of administration.	
*I.V.	4
*Oral.	0
*I.M.	0
- Duration of administration.	
<10 days.	1
>10 days.	3
Cases + ve by anaerobic culture.	2
Cases + ve by EIA.	4
Cases + ve by C.C.A.	4
Cases + ve by three tests.	2

Table (6) : **Incidence rate of toxigenic *C.difficile* as detected by EIA and cell culture assay (C.C.A) among the studied groups**

Group	Group I (40)		Group II (20)		Group III (20)		P.value
	No.	%	No.	%	No.	%	
- Total toxigenic.	4	10	0	0	0	0	0.12 (NS)
- +ve by EIA alone.	4	10	0	0	0	0	
- +ve by C.C.A. alone.	4	10	0	0	0	0	
- + ve by both tests.	4	10	0	0	0	0	

This table shows that :

- In group I : Incidence rate of toxigenic *C.difficile* was 10%.
- All cases +ve by EIA were also +ve by C.C.A.
- In group II & III : All stool specimens were negative for toxin assay.
- P.value = 0.12 (Non-significication) (NS).

Table (7) : **Results of the three tests used for detection of *C.difficile.***

Variable	Total toxigenic (4)	
	No.	%
+ ve by anaerobic culture.	2	50
+ ve by both EIA & C.C.A	4	100
+ ve by the three tests.	2	50

This table shows that :

- Out of the 4 total toxigenic *C.difficile* cases detected by EIA & C.C.A, only 2 cases were also positive by culture, however, the other 2 cases were negative.

Fig. (3) : Results of the three tests used for detection of C.difficile

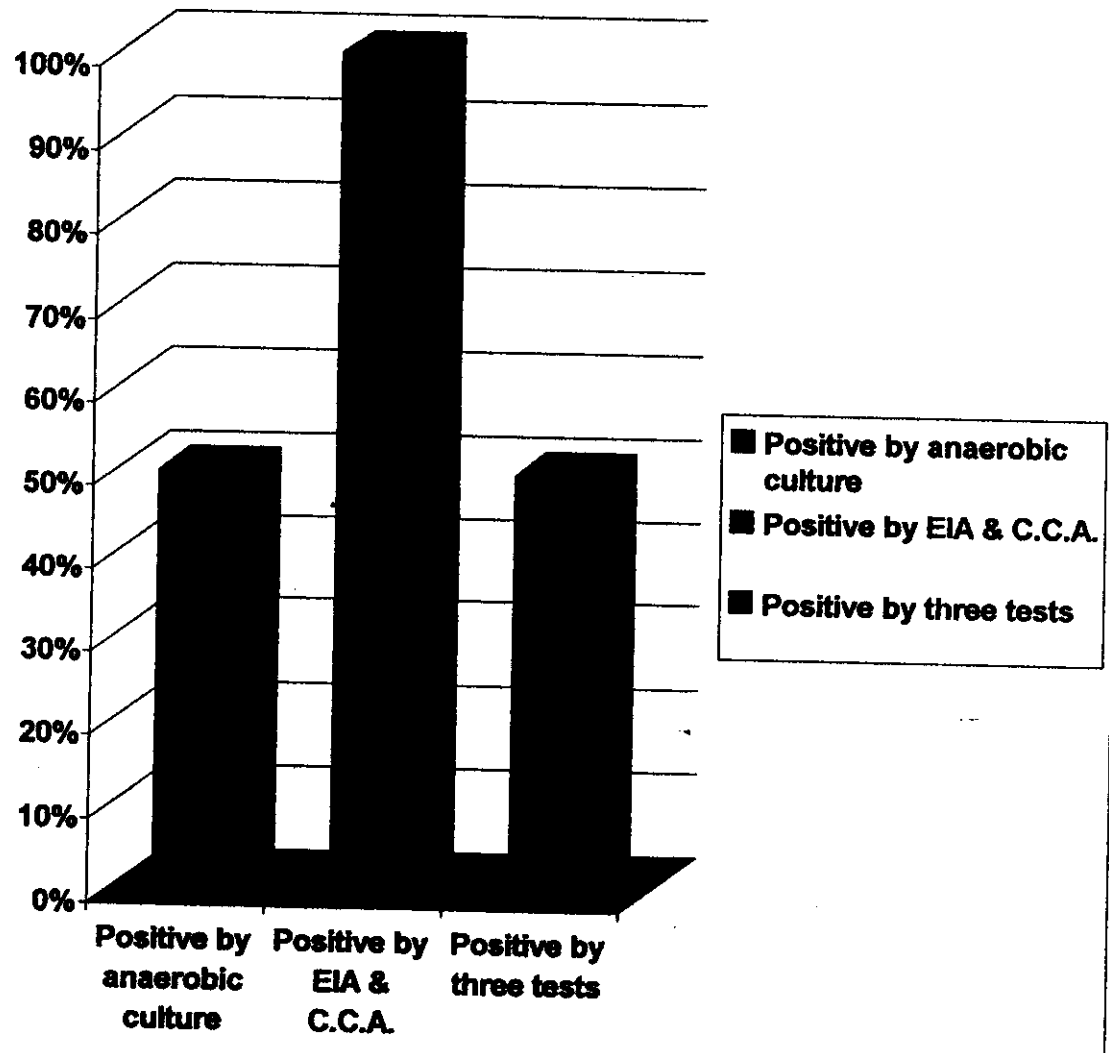

Table (8) : **The incidence rate of toxigenic *C.difficile* in the hospitalized diarrheal patients (group I) according to epidemiological parameters.**

Variable	Total No.. (40)	+ve cases (4)		-ve cases (36)		P.value
		No..	%	No..	%	
Sex						
- Male	30	1	25	29	80.6	0.04 (S)*
- Female	10	3	75	7	19.4	
Mean age ± SD	51.7 ± 19.9	58.2 ± 15.1		39.1 ± 13.9		<0.05 (S)*

This table shows :

- A higher incidence rate of toxigenic *C.difficile* in the stool samples of female patients (75%) than in that of male patients (25%) and this difference is statistically significant. P. value = 0.04 (Significant) (S).
- Mean age of 58.2 ± 15.1 for the +ve cases. P.value < 0.05 (Significant) (S).

Table (9) : **The incidence rate of toxigenic *C.difficile* in the hospitalized diarrheal patients (group I) according to different clinical manifestations.**

Variable	Total No. (40)	+ve cases (4)		-ve cases (36)		P.value
		No.	%	No.	%	
Manifestations :						
- Fever.	15	4	100	11	30.6	0.006 (HS)**
- Abdominal pain.	10	4	100	6	16.7	0.0002 (HS)**

This table shows that :

- Fever is the presenting manifestation in all +ve cases (100%) with p.value = 0.006 (Highly significant) (HS).
- Abdominal pain is the presenting symptom in all +ve cases (100%) with p.value = 0.0002 (Highly significant) (HS).

Table (10) : **Characteristics of diarrhea in +ve and –ve cases of the hospitalized diarrheal patients (group I).**

Variable	Total No. (40)	+ve cases (4)		-ve cases (36)		P.value
		No.	%	No.	%	
Duration :						
- 1-5 days.	30	1	25	29	80.56	0.014 (S)*
- > 5 days.	10	3	75	7	19.44	
Frequency :						
-<10 motions/ day.	35	1	25	34	94.4	0.0001 (HS)**
->10 motions/day.	5	3	75	2	5.6	
Onset in relation to antibiotic therapy :						0.27 (NS)
- 1-10 days.	37	3	75	34	94.4	
- >10 days.	3	1	25	2	5.6	

This table shows :

- A higher incidence rate of toxigenic *C.difficile* in stool samples of hospitalized diarrheal patients suffering from diarrhea > 5 days than patients suffering from diarrhea 1-5 days, and this difference is statistically significant. P. value = 0.014 (S).
- A higher incidence rate of toxigenic *C.difficile* in stool samples of hospitalized diarrheal patients suffering from high frequency of diarrhea >10 motions/day than those suffering from diarrhea 1-10 motions/day and this difference is statistically highly significant P. value = 0.0001 (HS).
- A higher incidence rate of toxigenic *C.difficile* in stool samples of hospitalized diarrheal patients starting diarrhea 1-10 days after antibiotic therapy than patients starting it > 10 days after antibiotic therapy and this difference is statistically insignificant. P. value = 0.27 (NS).

Table (11) ; **The incidence rate of toxigenic *C.difficile* in the hospitalized diarrheal patients (group I) according to the duration of hospital stay.**

Variable	Total No.. (40)	+ve cases (4)		-ve cases (36)		P.value
		No.	%	No.	%	
Duration of hospital stay :						
- 3-10 days.	25	1	25	24	66.7	0.13 (NS)
- >10 days	15	3	75	12	33.3	

This table shows :

- A higher incidence rate of toxigenic *C.difficile* in the stool samples of hospitalized diarrheal patients stayed in the hospital > 10 days (75%) than patients stayed 3-10 days (25%) and this difference is statistically insignificant. P. value = 0.13 (NS).

Fig. (4) : The percentage of toxigenic *C.difficile* in the hospitalized diarrheal patients (group I) according to the duration of hospital stay.

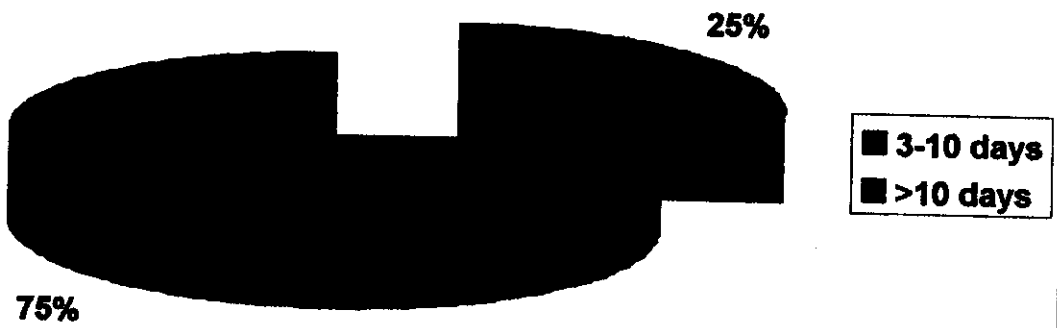

Table (12) : **The incidence rate of toxigenic *C.difficile* in the hospitalized diarrheal patients (group I) according to the underlying diseases**

Variable	Total No. (40)	+ve cases (4)		-ve cases (36)		P.value
		No.	%	No.	%	
Underlying diseases :						
- Malignancies.	15	2	50	13	36.1	0.48 (NS)
- Liver diseases.	5	1	25	4	11.1	0.42 (NS)
- Abdominal surgeries	5	1	25	4	11.1	0.42 (NS)
- Renal diseases.	5	0	0	5	13.9	0.57 (NS)
- Medical diseases.	5	0	0	5	13.9	0.57 (NS)
- P.U.O.	5	0	0	5	13.9	0.57 (NS)

This table shows that :

- The hospitalized diarrheal patients with malignant diseases had the highest rate of toxigenic *C.difficile* (50%) followed by patients with abdominal surgeries (25%) and patients with liver diseases (25%).

Fig. (5) : The percentage of toxigenic *C.difficile* in the hospitalized diarrheal patients (group I) according to the underlying diseases

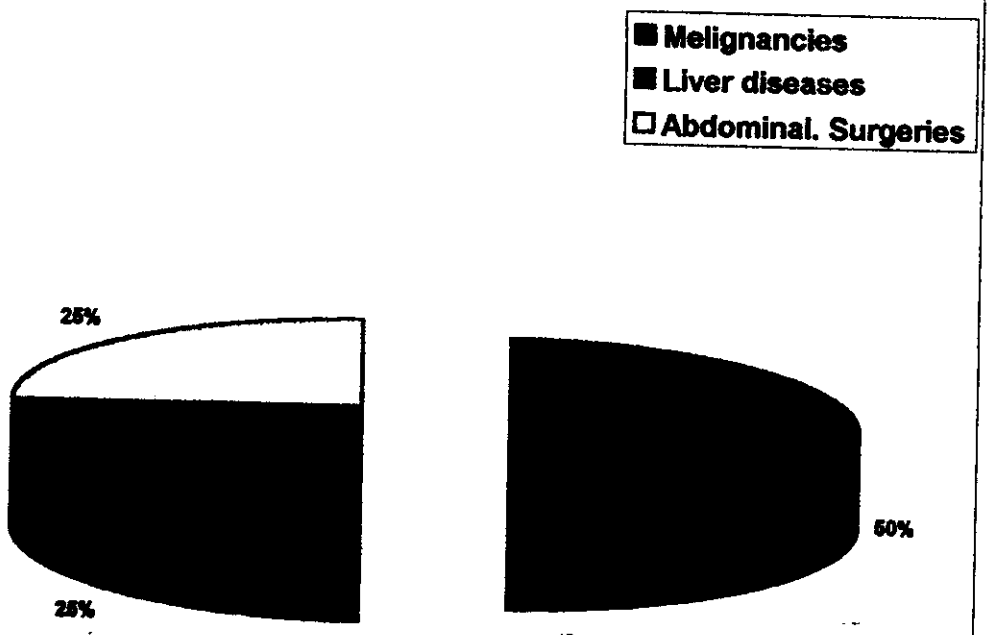

Table (13) : **The incidence rate of toxigenic *C.difficile* in the hospitalized diarrheal patients (group I) according to tube feeding.**

Variable	Total No. (40)	+ve cases (4)		-ve cases (36)		P.value
		No.	%	No.	%	
Tube feeding	10	3	75	7	19.4	0.014 (S)*

This table shows :

- Tube feeding was a significant feature in (75%) of +ve cases. P.value = 0.014 (Significant) (S).

Table (14) : **The incidence rate of toxigenic *C.difficile* in the hospitalized diarrheal patients (group I) according to antibiotic therapy**

Variable	Total No. (40)	+ve cases (4)		-ve cases (36)		P.value
		No.	%	No.	%	
Type of antibiotic :						
-Cephalosporins.	20	3	75	17	47.2	0.75 (NS)
-Ampicillins.	12	1	25	11	30.6	0.65 (NS)
-Clindamycins.	4	0	0	4	11.1	0.64 (NS)
-Macrolides.	4	0	0	4	11.1	0.64 (NS)
Rout of administration :						
- Oral.	5	0	0	5	13.9	0.57 (NS)
- I.V.	28	4	100	24	66.7	0.22 (NS)
- I.M.	7	0	0	7	19.9	0.44 (NS)
Duration of administration :						
- 3-10 days.	25	1	25	24	66.7	0.57 (NS)
- >10 days.	15	3	75	13	36.1	0.16 (NS)

This table shows that :

- The hospitalized diarrheal patients receiving cephalosporins had the highest rate of toxigenic *C.difficile* (75%) followed by ampicillin (25%).
- All +ve cases (100%) received antibiotics intravenously.
- A higher incidence rate of toxigenic *C.difficile* in the stool samples of hospitalized diarrheal patients received antibiotics > 10 days (75%) than patients received antibiotics for 3-10 days (25%).

Fig. (6) : The percentage rate of toxigenic C.difficile in the diarrheal patients (group I) according to antibiotic therapy

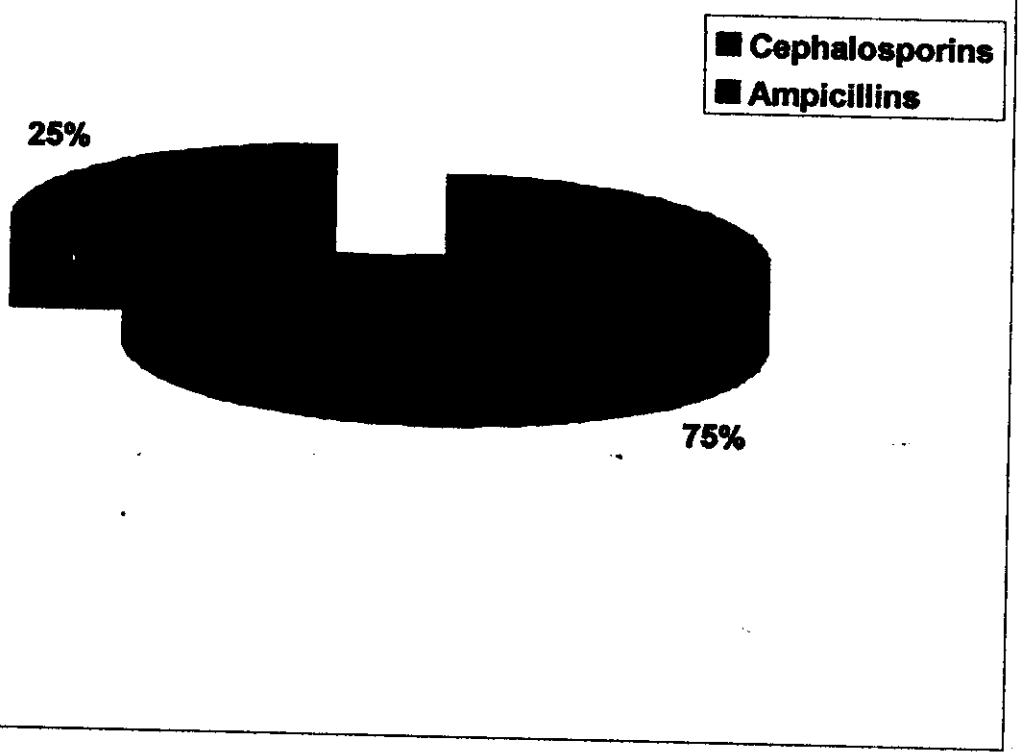

Table (15) : **Data of total +ve cases according to different parameters (total No. 4)**

Data	No. of +ve cases
Epidemiological data :	
- Age (mean age ± SD).	58.2 ± 15.1
- Sex :	
* Male.	1
* Female.	3
Clinical data :	
- Presence of fever and abdominal pain.	4
- Features of diarrhea :	
*Duration of diarrhea.	
<5 days.	1
>5 days.	3
*Frequency of diarrhea.	
<10 motions/ day.	1
>10 motions/day.	3
*Onset of diarrhea in relation to antibiotic.	
1-10days.	3
>10 days.	1
- Underlying disease :	
*Malignancies	2
*Abdominal surgery.	1
*Liver diseases.	1
*Renal diseases.	0
*Medical diseases.	0
*P.U.O. diseases	0
- Tube feeding.	3
- Duration of hospital stay.	
<10 days.	1
>10 days.	3
Antibiotic therapy :	
- Type of antibiotic.	
*Cephalosporins.	3
*Ampicillin.	1
*Clindamycins.	0
*Macrolides.	0
- Rout of administration.	
*I.V.	4
*Oral.	0
*I.M.	0
- Duration of administration.	
<10 days.	1
>10 days.	3
Cases + ve by anaerobic culture.	2
Cases + ve by EIA.	4
Cases + ve by C.C.A.	4
Cases + ve by three tests.	2

DISCUSSION

C.difficile is the most common cause of nosocomially aquired intestinal infections, affecting virtually all cases of PMC and up to 20% of cases of AAD ***(Pochapin, 2000).***

The pathologic findings associated with this infection are believed to be caused by two large exotoxins, toxin A (enterotoxin) and toxin B (cytotoxin) ***(Giannasca et al., 1999).***

Most cases of CDAD are associated with antibiotic therapy that alters the fecal flora, allowing overgrowth of *C.difficile* with production of its toxins ***(Brar and Surawicz, 2000).***

Overgrowth of toxigenic strains may result in a wide spectrum of diseases, which include, in increasing order of severity, asymptomatic carriage, self-limiting diarrhea, PMC and fulminant colitis which may result in toxic magacolon and/or intestinal perforation ***(Cleary, 1998).***

Symptomatic and asymptomatic patients are the primary reservoirs of and sources for environmental contamination ***(Hanna et al., 2000).***

Environmental contamination and carriage of the organism on the hands of hospital staff has been documented to transmit the infection ***(Fekety and Shah, 1993).***

Recurrence is a common sequela of *C.difficile* infection that may increase morbidity, costs and antimicrobial resistance. This problem

make the diagnosis and treatment of CADA a necessary policy in any hospital ***(DoAn et al., 1998).***

The diagnosis of CDAD was considered correct if all of the following conditions were met : the patients had received an antibiotic during the previous four weeks, subsequently developed diarrhea or liquid stool, the diarrhea had ceased after withdrawal of antibiotic or after the administration of vancomycin, or metronidazole ***(Riley et al., 1995)*** and stool samples were positive for isolation and toxigenicity studies ***(Schue et al., 1994).***

Metronidazole and vancomycin are the main agents used to treat patients with CDAD ***(Al-Eidan et al., 2000).***

The aim of this work was to determine the prevalence of *C.difficile* in stool samples from patients suffering from diarrhea after prolonged use of antibiotics, to evaluate the role of *C.difficile* in antibiotic associated diarrhea and to determine which antibiotics are most often responsible.

This study was conducted on 80 subjects divided into three groups:

-Group I (Hospitalized diarrheal patients) :

Included 40 patients suffering from AAD, their ages ranged from 18-90 years and stayed in the hospital from 3-14 days.

-Group II (Hospitalized non-diarrheal patients) :

Included 20 patients not suffered from diarrhea, their ages ranged from 18-90 years and stayed in the hospital from 3-14 days.

-Group III (Healthy control) :

Included 20 normal apparently healthy subjects. Their ages ranged from 18-60ys.

The stool samples were taken from patients and controls and tested by anaerobic culture, EIA and cell culture assay.

In the present study, 10% of hospitalized diarrheal patients with AAD (Group I) were demonstrated to have *C.difficile* infection responsible for their diarrheal episodes.

Fekety et al., (1981) were the first to document that *C.difficile* was responsible for 15% of cases of AAD, ***Viscidi et al., (1981)*** reported that *C.difficile* was responsible for 25% of cases of AAD seen in their research. A range of 15-25% was reported by ***Vogel, (1995). Gorenek et al., (1999)*** reported that 20.1% of AAD are due to *C.difficile*. In a more recent study by ***Titov et al., (2001)***, *C.difficile* was isolated from 1.8% of patients with AAD.

The variation in the incidence of CDAD along the last two decades may be attributed to better recognition of the organism, the degree of restriction of antibiotic use among different centers, as well as to factors that affect the rate of colonization ***(Mylonakis et al., 2001).***

Testing of the stool for toxin combined with cultural techniques had yielded discrepant results as reported by ***Brazier, (1993) and Riley et al., (1995).***

Results of the present study showed that out of the total toxigenic *C.difficile* cases detected by EIA and cell culture assay, only 50% were +ve by culture. The presence of toxin in the stool of patients group but with a negative culture can be explained either by recent antibiotic treatment or by failure of the methods used for isolation.

These results agreed with that of ***Sultana et al., (2000)*** who reported that only 60% of CDAD cases were positive by culture. They attributed this result to the difficulty of culture methods because of strict anaerobic nature of the organism.

In the current study, CCFA medium was used as a selective medium and blood agar medium was used as an ordinary medium after treatment with absolute alcohol for 1/2 hour at room temperature.

The ordinary medium showed an excellent results in comparing to CCFA, where all cases positive on CCFA showed also positivity on blood agar after alcohol shock.

The present study revealed that all cases who were positive by E1A (Tox A/B test), were also positive by cell culture assay.

Similar results were previously reported by ***Borriello et al., (1992)*** who reported that, most strains of *C.difficile* produce both toxins (A & B) under the same environmental conditions, however, rare strains behave as tox A-/B+ which are negative by tox. A specific E1As but are positive by tox A/B tests.

Lyerly et al., (1998) also stated that the correlation of tox A/B test with cell culture was 99.0%. So, tox A/B test is suitable as diagnostic aid for *C.difficile* diseases because it correlate well with cell culture and detect isolates that may be missed with tox A specific E1As.

In the present study, all cases of hospitalized non-diarrheal group (Group II) were negative for *C.difficile* by both culture and toxin assay.

This result disagreed with that of ***Johnson et al., (1990)*** who reported that asymptomatic *C.difficile* colonization in hospitalized patients is much more frequent and detected in 10-30% of patients.

The present study also revealed that, all subjects of healthy control group (Group III) were negative for *C.difficile* by both culture and toxin assay.

In the study done by ***Riley et al., (1995)*** they found that 0.47% were positive for *C.difficile* in apparently healthy individuals.

This result disagreed with the result of ***Viscidi et al., (1981)*** who stated that approximately 3% of healthy adults harbour *C.difficile* organism as a component of the normal flora. Also, ***Frost et al., (1999)*** found that, *C.difficile* can be isolated from stools of 2-5% of adult healthy volunteer subjects.

The present study revealed a higher incidence rate of toxigenic *C.difficile* in the stool samples of hospitalized diarrheal patients (Group I) aged > 50 years old than in patients aged < 50 years old.

The result of this study agreed with that of ***Aronsson et al., (1985)*** who reported that, the incidence of *C.difficile* toxin positive stools is 20 to 100 times greater for persons over 50 years old than for those 10 to 20 years of age. These data suggest that, the aging process is associated with increasing susceptibility to colonization, toxin production and disease by *C.difficile*, although the mechanism is not known.

Similar results were also reported by ***Al-Eidan et al., (2000)*** who found that, the increase of age is an important risk factor for *C.difficile* infeciton.

The present study also revealed, a higher incidence rate of CDAD among female hospitalized diarrheal patients of (Group I), than in male patients of the same group.

This result agreed with that of ***Brown et al., (1990) and Anade et al., (1994)*** who showed that a large proportion of CDAD patients are female; however, the statistical difference was not mentioned.

Regarding age and sex, the results of the present study disagreed with that of ***McFarland et al., (1990)*** who reported that, risk factor analysis showed no association between *C.difficile* infection and sex and age.

Regarding the incidence rate of toxigenic *C.difficile* among hospitalized diarrheal patients according to the duration of hospital stay, the result of the present study showed, a higher incidence rate of toxigenic *C.difficile* in the stool samples of patients stayed more than 10 days than patients stayed 3-10 days.

The result of the present study agreed with that of ***Macgowan et al., (1995) and Riley et al., (1995)*** who reported, a higher incidence of CDAD among patients who have had a relatively long duration of hospitalization.

Also, this result agreed with that of ***Al-Eidan et al., (2000)*** who reported that adult patients with CDAD remained in the hospital for almost 2 weeks longer than adult patients without CDAD.

In the present study, tube feeding was a risk factor for aquisition of CDAD.

Similar results were reported by ***Bliss et al., (1998)*** who found that hospitalized tube-fed patients especially those receiving post-pyloric tube feeding are at greater risk for aquisition of *C.difficile* and the development of CDAD than other hospitalized non-tube fed patients.

Regarding the risk factors associated with CDAD, the highest incidence rate of toxigenic *C.difficile* was found among hospitalized diarrheal patients with malignant diseases (50%) followed by patients with gastrointestinal surgeries (25%) and patients with hepatic encephalopathy (25%).

The results of this study are in agreement with the results of ***Heard et al., (1986)*** who found that *C.difficile* is a common pathogen among immuno-compromised patients. ***Reinke and Messick, (1994)*** also stated that exposure to antineoplastic chemotherapeutic agents alter the normal gastrointestinal flora, allowing overgrowth of *C.difficile* with production of its toxins. ***Sriuranpong and Voravud, (1995)*** concluded that moderate

to severe diarrhea in cancer patients after chemotherapy should alert the physician to be aware of a potential fatal complication caused by *C.difficile* infection.

The results of the present study agreed with that of ***Yukari et al., (1995)*** who reported that there is a strong association between abdominal surgery and the presence of CDAD, the incidence was 14%.

In a previous study done by ***ITo et al., (1997)***, the rate of diagnosis of *C.difficile* was 20% in patients with liver cirrhosis. These observations in agreement with our study and indicate that *C.difficile* is of clinical importance in development of hepatic encephalopathy either in pre-coma or comatozed patients.

In this study, cephalosporins (75%) and ampicillins (25%) were the agents most often associated with CDAD. However, it would be premature to rank these antibiotics in this order as agents causing CDAD and so, we agree with openion of ***Riley et al., (1995)*** who reported that the frequent association of certain antibiotics with CDAD might just reflect their frequent administration in the study hospitals and not necessarily to their ability to cause CDAD.

The results of the present study agreed with that of ***Walker et al., (1993)*** who stated that, the use of antibiotics particularly cephalosporins is a significant risk factor for aquisition of *C.difficile* infection. Other researches by ***Dodson and Borriello (1996)*** found that the cephalosporins, penicillins and aminoglycosides being particularly implicated in production of CDAD. ***Al-Eidan et al., (2000)*** also reported that published association between certain antimicrobials and CDAD

have been variable, but the highest association had been found among patients given cephalosporins. In their study, 84% of cases were received cephalosporins.

The results of this study disagreed with that of ***Gerding et al., (1986)*** who stated that clindamycin had the highest risk of antibiotics for the development of CDAD. ***Williams et al., (1994)*** reported an outbreak of CDAD characterized by the wide spread use of clindamycin in the hospital and the single intervention responsible for stopping of this outbreak was the removal of this agent. In a study done by ***Kelly et al.,(1994)***, they found that amoxicillin, clindamycin and quinolones were the agents most often responsible for CDAD.

The current study revealed that, 100% of CDAD were received antibiotics intravenously. This is in agreement with ***Al-Eidan et al., (2000)*** who found that CDAD had been reported after oral, parentral and even topical administration of antimicrobial therapy.

In the present study, fever and abdominal pain were the presenting manifestations in all cases of CDAD, each constitute a factor favoring the diagnosis of *C.difficile* positivety. These predictors for *C.difficile* positivity were previously reported by other investigators ***(Gerding et al., 1995 Yukari et al., 1995).***

However, this result disagreed with the result of ***Al-Eidan et al., (2000)*** who showed that fever and abdominal pain could not be solely attributed to CDAD as result of concurrent infections in many patients.

The present study also revealed that, duration of diarrhea 5 days or more and frequency of diarrhea 10 motions/day or more, were significant diarrheal characters in all positive cases.

Similar results were previously reported by ***Yukari et al., (1995).***

Regarding the onset of diarrhea in relation to antibiotic treatment, this study revealed a higher incidence rate of toxigenic *C.difficile* in patients suffering from diarrhea starting within a period of 1-10 days of antibiotic therapy than those with diarrhea starting> 10 days of antibiotic therapy.

This result agreed with that of ***Fekety., (1995)*** who reported that the onset of diarrhea is usually noted during antibiotic treatment ranging from 24 hours up to 4-9 days after starting antibiotics. However, in up to one third of patients, diarrhea does not begin until up to 6 weeks after antibiotic treatment has been discontinued.