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Bacteria translocation related to disease severity pre and post treatment hepatitis C virus

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Abstract

Hepatitis C virus (HCV) infection is complicated by microbial translocation throughout the movement of living bacteria or their endotoxins from the intestinal lumen to the mesenteric lymph nodes and other extra-intestinal locations. The present study aimed to evaluate bacterial translocation in patients with HCV infection pre and post-treatment. Results showed that HCV titer was high in pretreated patients and low in post-treated patients, the isolated bacteria were identified according to morphological cultures, the VITEK-2-system, and antibiotics. It was found that 76% of pretreated patients gave bacterial isolates and divided them according to symptoms; 16% showed mild symptoms, 49.33% showed moderate symptoms and 10.66% showed severe symptoms. The isolated bacteria were mostly multi-drug resistant. The frequency percent of trans-located bacteria was 29.33% for Gram-positive cocci bacteria included (25.33% *Streptococcus spp* and 4% *Staphylococcus spp*), and 46.66% for Gram-negative bacilli bacteria included (33.33% *E.Coli* and 13.33% *Klebsiella spp*). 6.66% of post-treated patients gave bacterial growth as 2.66% showed moderate symptoms, and 4% showed severe symptoms. The percent of bacteria was 5.33% for Gram-positive cocci bacteria included (4% *Staphylococcus spp* and 1.33% *Streptococcus spp*), and 1.33% for Gram-negative bacilli bacteria. According to the studied patients, Results showed a significant difference between pre and post-treated patients as the highest percentage of bacterial growth was found in the pretreated patients and more bacterial isolates were from patients with severe symptoms while most samples that gave no growth were from patients who showed mild symptoms.

Key words: bacterial translocation- hepatitis C virus infection- HCV treatment.

Introduction:

Patients with HCV infection are an increasing problem in Egypt in terms of economic and health care costs. Bacterial infection is frequent in most patients with HCV and reflects the most common cause of hospitalization. Recurrent bloodstream infection considers one of the most adverse infections associated with HCV (Bunchornatavakul *et al.*, 2016). Bacterial translocation is defined as the migration of micro-organisms from the gastrointestinal lumen (GL) to the mesenteric lymph nodes (MLN) and extra-intestinal sites, it considers a frequent phenomenon that leads to the presence of bacterial DNA circulating in the blood of a significant number of patients with HCV infection (Hasa *et al.*, 2022) and (Zafari *et al.*, 2022).

Gram-negative bacteria from the family Enterobacteriaceae (including *E.coli* and *Klebsiella spp*), *Enterococci*, and other *Streptococci* species are the most successful at bacterial translocation to the mesenteric lymph nodes. Anaerobic species, on the other hand, only translocate infrequently, and they have been shown to inhibit the growth of aerobic species with higher translocation capability (Vergalli *et al.*, 2020).

HCV infection was associated with markers of gut macrobiotic translocation, which in turn is thought to lead to immune activation and disease progression in HCV (Reid *et al.*, 2018). Bacterial translocation was shown to be related to the stage of liver failure and is more remarkable in advanced liver diseases (Tribicka *et al.*, 2021).

Egypt is one of the most countries affected by HCV. The Egyptian health issues showed that 14.7% of people aged 15-60 years had an active hepatitis infection in 2009 (Metwally *et al.*, 2021). And that decreased to 7% in 2005. Egypt developed a national strategy to face this challenge and to control HCV infection. This strategy was made up of six main objectives of control and prevention: improving blood safety, health education to communities and providers, care and treatment, infection control, surveillance, and hepatitis B vacation (Shahid *et al.*, 2021).

The study aimed to estimate bacterial translocation by the culture-based method in HCV status pre and post-treatment and define the trans-located bacteria with suitable antibiotics.

Material and Methods

Study design and setting

This study was done during the period from August 2020 to December 2022.

Study Participants

This study was conducted on 150 patients suffering from HCV infection in the Fever, Ophthalmology, and the University hospitals belonging to benha. All patients were given a full medical history, and HCV was detected based on symptoms, serologically by ILFST, ELISA, and molecular by quantitative RT-PCR in 150 serum samples collected from 75 patients pretreatment and 75 patients post-treatment. Patients with HCV infection mostly were asymptomatic but if they showed symptoms it usually like fatigue, visual dysfunction, exhaustion, nausea, fever, and muscle aches, and appearing of these symptoms depends on the disease's severity. Patients were divided according to disease severity into three groups: mild, moderate, and severe symptoms.

Inclusion criteria

The patient group included 73 males and 77 females and their ages ranged from 20 to 60 years old. Patients enrolled in each group were entirely selected so that they were matched in symptoms that would affect bacterial translocation.

Process

Full history data was taken including sex, age, presence of viral infection or not, and symptoms like abdominal pain, fatigue, fever, loss of appetite, Vomiting, and jaundice; Receiving treatment or not.

Specific investigation for the detection of bacteria was done including bacterial cultures, the definition of bacteria by physiological characteristics, and antibiotics resistance by the VITEK-2-system.

Methods:

Collecting specimen: All patients were given ten milliliters of venous blood through peripheral venipuncture under aseptic conditions, Three milliliters of venous blood were drawn in a lithium heparin tube then put in a water bath at 37 °C for 30 minutes then centrifuged at 100 r.p.m for 10 minutes to separate blood into three layers Plasma, Buffy coat (BC) and erythrocytes then separate the buffy coat to be used for culture (Castillo *et al.*, 2019). The rest of the blood was drawn in a plain tube then put in a water bath at 37 C° for 30 minutes then centrifuged at 100 r.p.m for 10 minutes then the resultant serum was drawn to a vacuum plain tube and stored in the freezer at -20 C° for Hepatitis C markers and biochemical measurements.

Detection of Hepatitis C: HCV Abs was detected by rapid detection cards, immune-chromatography lateral-flow strip test (ILFST) according to (Poiteau *et al.*, 2016) using ABON rapid detection cards, and 3rd generation enzyme-linked immune-sorbent assay (ELISA) according to (Jalees *et al.*, 2010) using Anti HCV Elisa Version 4.0. HCV RNA was detected by qualitative and quantitative RT-PCR according to (Tashkandy *et al.*, 2007) using COBAS Ampliprep/COBAS Taqman HCV test.

Isolation of bacteria: bacteria were isolated from the buffy coat using the streaking method (Harley and Prescott, 2002) for culture. All samples were cultured on blood agar media and incubated at 37℃ for 48 hrs. (Holt *et al.*, 1994) After the incubation time each plate was studied and counted by Auto-Col – Fully automated colony counting system then purification of bacterial isolates by a single colony, all plates were taken and plated on selective media as MacConkey (Mossel *et al.*, 1962) Nutrient (Blakely and

Steward, 1964) and Chocolate agar media (Gunn, 1984) and incubated at 37 ℃ for 48 hrs.

Identification of bacteria by growth characters, Gram stain and the VITEK-2-system: Bacterial growth and the morphological characters of each plate were recorded in a table then types of bacteria depending on Gram stain and the VITEK-2system (Ligozzi *et al*., 2002) were recorded in a table, and types of the isolated bacteria and the suitable antibiotics were identified.

Statics analysis: Using Excel, the mean and the standard deviation values were determined for all results.

Results

Hepatitis C virus detection: HCV Abs was detected in patients by **1- ILFST** where in patients pretreatment 76% showed positive results and in patients post-treatment 100% showed positive results. **2- ELISA** where in patients pretreatment 80% showed positive results and 13.3% showed equivalent results and in patients post-treatment 93.3% showed positive results and 6.66% showed equivalent results **3- Q RT-PCR** where in patients pretreatment all of them were positive with mean values 251.811 ± 181.910 while patients post-treatment all of them were Negative less than 10 (Table 1 and 2).

Table (1): serological and molecular detection for HCV patients pretreatment according to locale, age, and sex.

Hospitals	Sex	Age	No of Positive results of		
			ILFST	ELISA	Quantitative PCR
University N=21	Males N=9	>20 (n=4)	6	4	4
		>45 (n=5)	3	4	5
	Females N=12	>20 (n=7)	5	5	7
		>45 (n=5)	6	4	5
Fever N=39	Males N=18	>20(n=10)	4	9	10
		>45 (n=8)	9	7	8
	Females N=21	>20 (n=9)	7	8	9
		>45 (n=12)	7	7	12
Ophthalmology N=15	Males N=8	>20 (n=5)	4	3	5
		>45 (n=3)	1	3	3
	Females N=7	>20 (n=5)	2	4	5
		>45 (n=2)	3	2	2
Total	75	---	57	60	75

Table (2): serological and molecular detection for HCV patients post-treatment according to locale, age, and sex

Hospitals	Sex	Age	No of Positive results of		
			ILFST	ELISA	Quantitative PCR
University N=20	Males N=10	>20 (n=4)	4	4	0
		>45 (n=6)	6	5	0
	Females N=10	>20 (n=5)	5	5	0
		>45 (n=5)	5	5	0
Fever N=40	Males N=21	>20 (n=9)	9	9	0
		>45 (n=12)	12	11	0
	Females N=19	>20 (n=7)	7	7	0
		>45(n=12)	12	10	0
Ophthalmology N=15	Males N=7	>20 (n=4)	4	3	0
		>45 (n=3)	3	3	0
	Females N=8	>20 (n=2)	2	2	0
		>45 (n=6)	6	6	0
Total (75)	---	---	75	70	0

According to the disease severity; patients pre and post-treatment were divided into three groups: from pretreated patients, 17 showed mild symptoms such as fever, vomiting, and loss of appetite with disease severity of 4.33%, 47 showed moderate symptoms as fatigue with disease severity 13.33%, and 11 showed severe symptoms as liver cell failure, jaundice, dark yellowed urine and taking longer of bleeding to stop with disease severity 39.83%. From post-treated patients 9 patients showed mild symptoms as Fatigue, Headache, and muscle pain with a disease severity of 5.06%, 6 patients showed moderate symptoms such as anemia with a disease severity of 6.4%, and 5 patients showed severe symptoms such as cirrhosis and fibrosis with disease severity 6.6% while the other 55 patients showed no symptoms related to HCV infection.

Trans located bacteria detection

Bacterial detection in pre and post treated HCV patients.

The obtained results found significant differences between bacterial growth in HCV patients pre and post-treatment. Out of seventy-five pretreated patients with different HCV titers, there were fifty-seven patients who gave detected bacteria, including 35 of which were short bacilli Gram-negative bacteria, 22 were cocci Gram-positive bacteria and 18 showed no detectable bacteria. On the other hand out of seventy-five post-treated HCV patients, there were only five samples gave detectable bacteria, including four cocci Gram-positive bacteria, one bacillus Gram-negative bacteria, and the other 70 patients showed no growth of detected bacteria.

Patients pretreatment was divided into three groups according to their HCV titer of Q-PCR Low titer (10 – 10^5) IU/ml, Moderate titer (10^5 – 10^6) IU/ml, and high titer (>10^6) IU/ml. and according to detected bacteria as patients with cocci bacterial growth (2.1.log2 colony per plate), patients with bacilli bacterial growth (2.9.log2 colony per plate), and patients with no bacterial growth (less than 20 colonies per plate). As shown in (Tables 3 and 4) and (Figure 1).

Table (3): Bacterial detection in HCV patients pretreatment.

Hospitals	ILFST Positive	PCR titer		Bacterial growth log CFU/ml		
				Cocci	Bacilli	No growth
University hospital (N= 21)	20/21 95.8 %	Low	2	2(2.5.log2)	5(2.4.log2)	3
		Moderate	16	2(1.9.log2)	4(2.5.log2)	1
		High	2	1(16.log2)	2(2.2.log2)	1
Fever hospital (N= 39)	27/39 69.2 %	Low	11	7(1.8.log2)	9(2.5.log2)	3
		Moderate	21	2(1.9.log2)	6(2.1.log2)	2
		High	7	3(2.4.log2)	4(2.3.log2)	3
Ophthalmology hospital (N= 15)	10/15 66.6%	Low	4	2(1.4.log2)	2(1.9.log2)	4
		Moderate	11	1(2.1.log2)	1(1.8.log2)	1
		High	0	2(2.7.log2)	1(2.5.log2)	1

Table (4). Bacterial growth in HCV patients Post treatment.

Location	ILFST Positive	PCR Negative <10 IU/ml	Bacterial growth log CFU/ml		
			Cocci (100-150) colony	Bacilli (100-150) colony	No growth < 20 colony
University hospital (N= 20)	20/20 100%	20/20 100%	2 1.5.log2	0	22
Fever hospital (N= 40)	40/40 100%	41/41 100%	2 1.7.log2	1 2.2.log2	38
Ophthalmology hospital (N= 15)	15/15 100%	10/10 100%	0	0	10

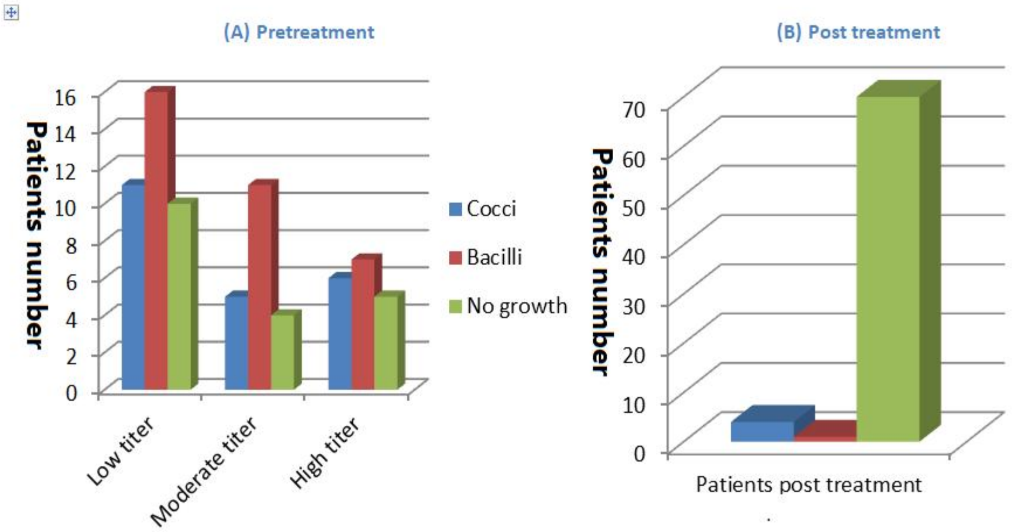

Figure (1): Histogram showing Bacterial growth in HCV patients (A) pretreatment and (B) post treatment according to q-PCR titer.

Bacteria associated with hepatitis C according to disease severity.

The identified bacteria among patients pretreatment were 70.5 % for patients with mild symptoms, 78.7 % for patients with moderate symptoms, and 72.7 % for patients with severe symptoms which could infer that as the bacterial growth increases the disease severity increase. Post-treated patients only showed bacterial growth in patients with severe and moderate symptoms while all patients with mild symptoms gave no bacterial growth which means that the presence of bacteria increases the symptoms and disease severity.

Bacterial growth in patients with HCV pretreatment varied between short rod bacilli Gram-negative bacteria (58.66%) and cocci Gram-positive bacteria (17.33%) and some gave no growth (24%).

According to these data short rod bacilli, Gram-negative bacteria were more associated with HCV patients pretreatment.

HCV patients post-treatment mostly showed no bacterial growth as only five patients gave bacterial growth varied between cocci Gram-positive bacteria (5.3%) and short rod Gram-negative bacteria (1.33%). These data are described in (Table 5) and (Figures 2 and 3).

There were significant differences in bacterial growth with patients post treatment as (93.33%) of patients gave no bacterial growth and the other (6.67%) who gave bacterial growth could be not related to the hepatitis C infection

Table (5): Bacterial count associated with HCV titer and disease severity.

	Symptoms	Disease severity	Cocci growth		Bacilli growth		No growth
			N of Patients	CFU/ml	N of Patients	CFU/ml	
Pretreatment patients N= 75	Mild n=17	4.33 %	3	175	9	200	5
	Moderate n=47	13.33 %	9	180	28	220	10
	Severe n=11	39.83 %	1	200	7	250	3
Frequency	N=75	----	13	555	44	670	18
Post treatment patients N= 75	Mild n=9	5.06 %	0	----	0	----	9
	Moderate n=6	6.4 %	2	100	0	----	4
	Severe n=5	6.6 %	2	200	1	125	2
	No symptoms =55	0 %	0	----	0	-----	55
Frequency	N=75	----	4	150	1	125	70

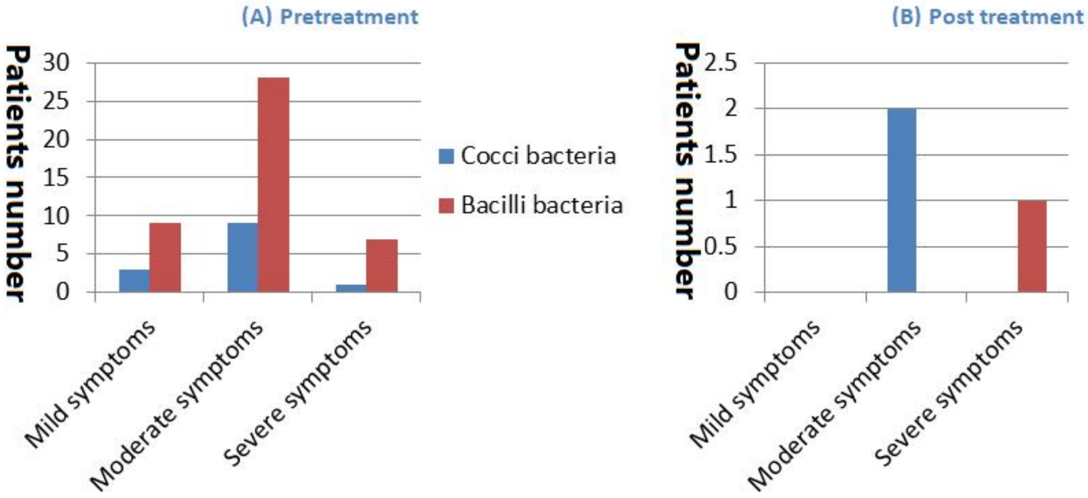

Figure (2): Histogram showing Bacterial growth according to disease severity for patients (A) pretreatment and (B) post treatment

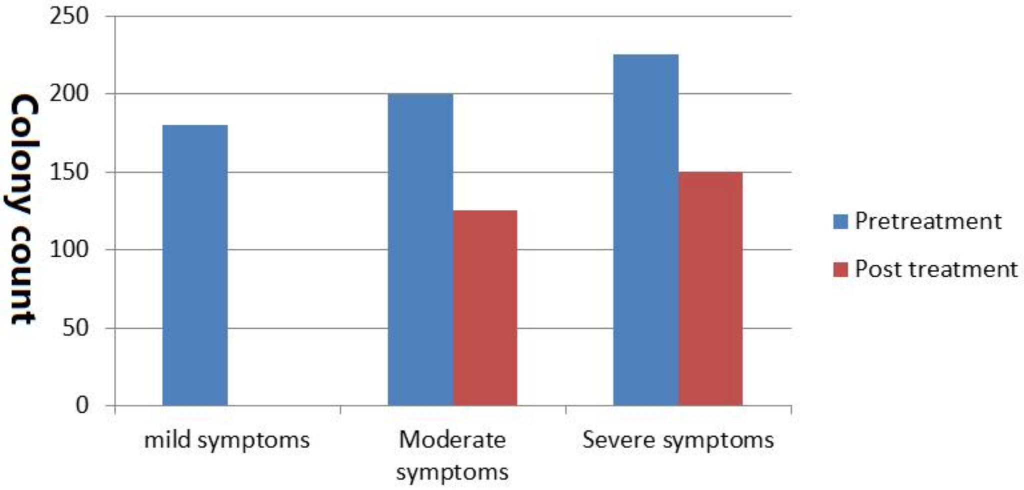

Figure (3): Histogram showing Bacterial count according to disease severity for patients pre and post treatment

Identification of bacteria: The isolated bacteria have been identified by the bacterial growth cultures with their cultivation properties and by the VITEK-2 system.

Each sample for patients pre and post-treatment were cultured on Blood agar media and incubated at 37℃ for 48 hrs. After incubation time the appeared colonies were counted by AutoCol – A fully automated colony counting system and stained by Gram stain Egyptian Diagnostic Media (EDM) to differentiate whether they were Gram positive or Gram negative and by seeing the slide under the microscope determine if they were cocci or bacilli bacteria. Then the predominant bacteria from each plate were isolated in different culture media such as Nutrient, Chocolate, and MacConkey agar media. According to the morphologic and microscopic characters, there were four types of predominant bacteria two cocci Gram-positive and two bacilli Gram-negative. The MacConkey agar media mostly gave growth with bacilli Gram-negative bacteria and gave no growth with cocci Gram-positive bacteria, Chocolate and Nutrient agar media mostly gave growth with both cocci Gram-positive and Bacilli Gram-negative bacteria. The bacterial growth on these different media is described on (Table 6) and (Figure 4).

Table (6): bacterial growth of patients pretreatment on different culture media.

	No. of plates that gave growth on		
	MacConkey agar	Chocolate agar	Nutrient agar
Bacilli G -ve bacteria	40/44	32/44	28/44
Cocci G +ve bacteria	0/13	12/13	10/13

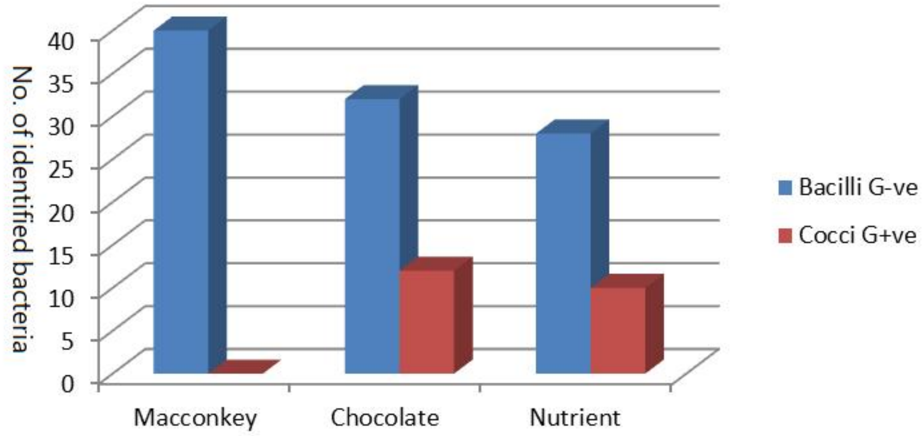

Figure (4): Histogram showing growth of bacteria in patients pretreatment on different media.

Frequency of common bacteria associated with HCV.

By using the VITEK-2-system and comparing with VITEK-2 system software, different types of bacteria could be determined from the plates of patients pre and post-treatment which include Gram-negative short rod bacilli bacteria like *E.coli* with probability (98.2%) and *Klebsiella spp* with probability (97.5%) and Gram-positive cocci bacteria like *Streptococcus spp* with probability (96.5%) and *Staphylococcus spp* with probability (97.8%) (Figure 5, 6, 7 and 8).

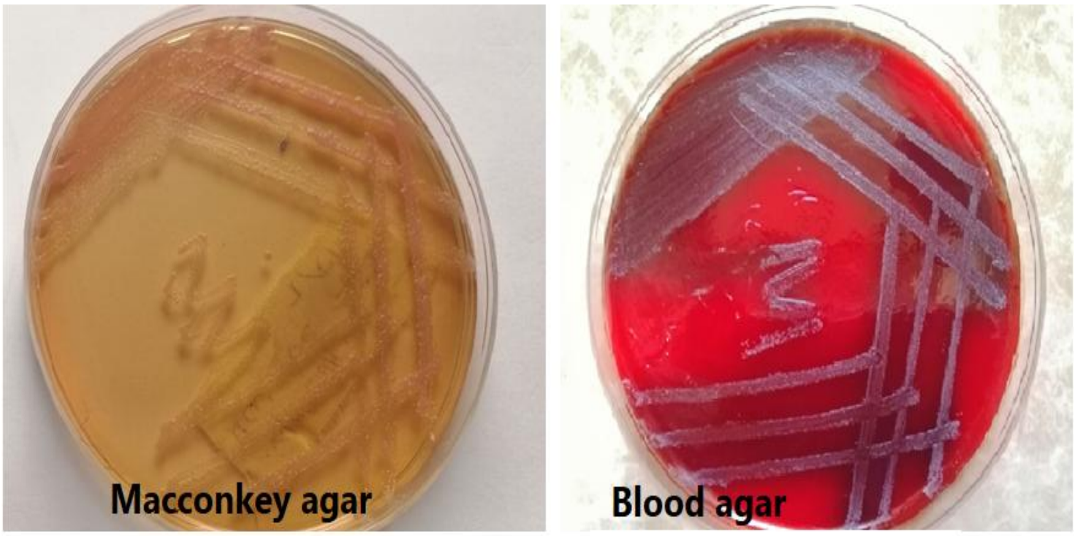

Figure (5): plate growth of *E.Coli* using blood and MacConkey agar media.

Figure (6): plate growth of *Klebsiella spp* in blood and MacConkey agar media.

Figure (7): plate growth of *Streptococcus spp* in blood and chocolate agar media

Figure (8): plate growth of *Staphylococcus* on blood and chocolate agar media

From the 57 patients pretreatment who gave bacterial growth, there were 30 patients gave growth of *E.Coli*, 14 patients gave growth of *Klebsiella spp*, 10 patients gave growth of *Streptococcus spp* and 3 patients gave growth of *Staphylococcus spp* and from the 5 patients post-treatment who gave bacterial growth, there were 1 patients gave growth of *E.Coli*, 1 patients gave growth of *Streptococcus spp*, and 3 patients gave growth of S*taphylococcus spp* (Figure 9).

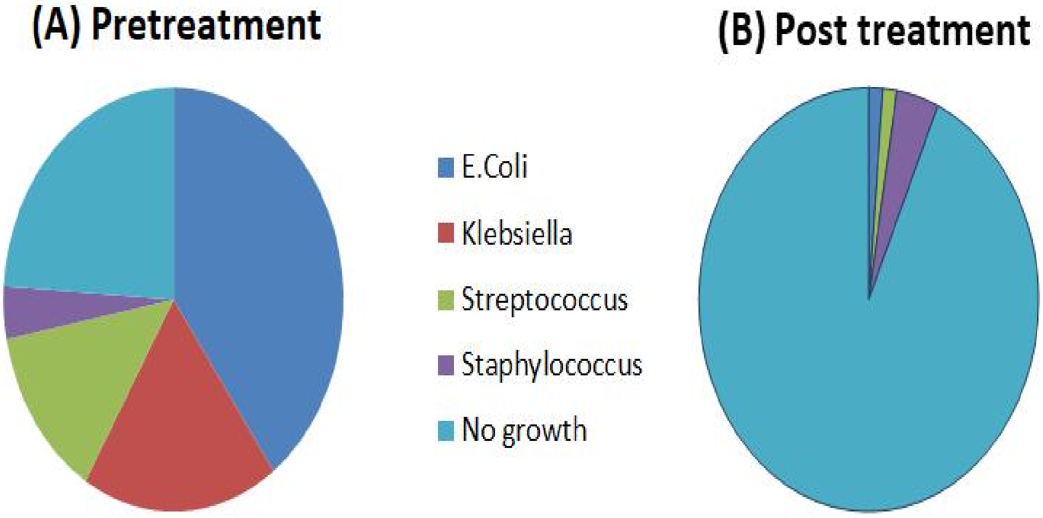

Figure (9): Histogram showing Bacterial growth in HCV patients (A) pretreatment and (B) post treatment

According to symptoms related to HCV infection in patients pretreatment, it was found that more bacterial isolates were from patients with severe symptoms while most samples that gave no growth were from patients who showed mild symptoms, from these data it can be concluded that bacterial growth increase as the disease severity increase (Table 7) and (Figure 10).

Table (7): Bacterial growth in HCV patients pretreatment associated with disease severity

Disease severity	Bacterial growth (n=57)					No growth (n=18)
	E.coli	*Klebsiella*	*Strepto-coccus*	*Staphylo-coccus*	Frequency	
Mild symptoms	6	3	1	0	10	10
Moderate symptoms	10	5	2	1	18	5
Severe symptoms	14	6	7	2	29	3

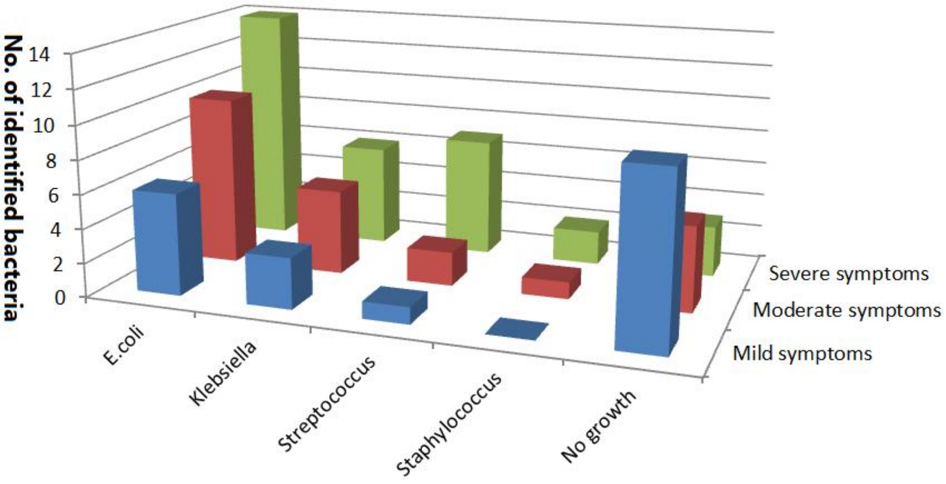

Figure (10): Histogram showing Bacterial growth in HCV patients pretreatment associated with disease severity

Antibiotics resistance: The identified bacteria were tested for antibiotics sensitivity by the VITEK-2-system and most of them were multi-drug resistant as described in **(Table 8).**

Table (8): Antibiotic sensitivity for the identified bacteria.

Antibiotics	**G + ve bacteria**				**G - ve bacteria**			
Pharmaceutical Name	***Staphylococcus***		***Streptococcus***		***E.Coli***		***Klebsiella***	
	MIC		**MIC**		**MIC**		**MIC**	
Ampicillin	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Gentamycin	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Ciprofloxacin	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Levofloxacin	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Nitrofurantoin	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Trimethoprime/ Sulfamethoxazole	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Tobramycin	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Cefepime	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Oxacillin	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Rifampicin	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Ceftazidim	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Ceftriaxone	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Amikacin	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Erythromycin	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]

Clindamycin	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Vancomycin	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Tetracycline	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]

Discussion

This study investigated the possibility of an association between microbiota and hepacivirus in Egyptian patients with hepatitis C, both before and after therapy, based on gender and age. This finding is supported by dates from a previous study. In a recent study, **(Honda *et al.*, 2021)** revealed bacterial translocation among patients with chronic hepatitis C, but it was unrelated to liver fibrosis stages or systemic inflammation.

In the current study, bacterial growth was detected in 150 patients with HCV infection, 75 pretreated patients (35 males and 40 females) and 75 post-treated patients (38 males and 37 females) with ages ranging from 20 to 60 years, collected from various hospitals in Egypt's El Qalyobia governorate.

Clinical symptoms, ILFST, ELISA, and Q-PCR were used to diagnose HCV infection. Bacterial growth was detected using Blood agar culture, Gram stain, and the VITEK-2-system.

Most infections of HCV are asymptomatic, despite elevations in liver enzymes level. According to **(Eric Dieperink et al., 2000)** about 30% of the people who newly acquired HCV infection develop symptoms, such as fatigue, loss of appetite, and jaundice.

Rapid detection cards based on the principle of immune chromatography were used to diagnose HCV Abs in patients' serum (**Gaiping *et al.*, 2009).** After the identification and characterization of HCV Abs, molecular assays such as ELISA and Q-PCR were used to detect, measure, and characterize the infecting virus, as confirmed **by (Selda, 2001)**.

Bacterial growth was isolated using cultured-based method as corroborated by old studies for example **(Holt *et al.*, 1994)**. The highest percentage of bacterial growth among patients with HCV infection was in patients pretreatment, especially with patients showed severe symptoms and high titer of Q-PCR followed by patients who showed moderate symptoms. This association agreed with **(Moriello *et al.*, 2023)** who showed that gut microbiota most likely plays a fundamental role in the etio-pathogenesis of liver disease and especially in its evolution.

Although the revolutionary impact of direct-acting antiviral therapy on HCV is already known, these therapies could have an even more significant indirect impact on the microbiota and the resulting liver conditions, this agreed with **(Abd El-hai *et al.*, 2021)** who showed that Bacterial translocation markers (bacterial DNA and endotoxin) are high in cirrhotic and HCV infected patients.

Results showed a significant difference in bacterial growth with HCV patients' pre and post-treatment, as pretreated patients showed a high growth percentage of bacteria despite post-treated patients who showed a very low percentage of bacterial growth, this agreed with (**Lattanzi *et al.*, 2018)** who showed that microbial translocation markers and T cell activation are increased in HCV-infected patients with liver fibrosis and decrease during direct-acting antiviral treatment.

The bacterial isolates were determined based on morphological, biochemical characteristics, and Gram stain which showed variable growth between short rod bacilli Gram-negative bacteria and cocci Gram-positive bacteria in patients pretreatment while most showed no bacterial growth in patients post-treatment as only five patients give bacterial growth one gave growth of short rod bacilli Gram-negative bacteria and the other four isolates were cocci Gram-positive bacteria, these data agreed with a recent study by **(Lopez-cortes *et al.*, 2018)** who described that there were decreases in microbial translocation markers after the eradication of HCV infection

The VITEK-2 system was used to identify the bacterial isolates and it was found that with HCV patients pretreatment the most predominant bacteria was *E.coli* that is corroborated by (**Moon *et al.*, 2019)** who found that, the increase in microbial markers (lipopolysaccharide) noted in HCV patients could be due to an increase in microbial translocation from the gut, and Lipopolysaccharide (LPS) is a component of the Gram-negative bacterial (*E.coli*).

Bacilli Gram-negative bacteria and cocci Gram-positive bacteria isolates were found in a high percentage of HCV patients pretreatment that is agreed with **(Twonsend *et al.*, 2021)** who estimates evidence of Gram-negative and Gram-positive bacteria species have been documented in blood, ascetic fluid, and liver tissue of HCV and cirrhotic patient.

The antibiogram profile and multiple antibiotics resistance (MAR) patterns were determined for the identified bacteria as *Escherichia coli, Klebsiella spp, Streptococcus spp*, and *Staphylococcus spp* and most of the isolates showed multiple antibiotics resistance (MAR). The antibiogram profile of isolates revealed Ciprofloxacin, and Levofloxacin as the most effective antibiotics against most isolated bacterial species while they are resistant to most other antibiotics this agreed with a very recent study (**Akande *et al.*, 2019)** showed that Ciprofloxacin was the most effective antibiotic against most isolated bacterial.

Conclusion

Bacterial translocation is higher in patients with HCV infection pretreatment than in patients post-treatment and increases with the disease severity.

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