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Bacterial antibiotics resistance pattern in patients with chronic hepatic disease and the effect of probiotics

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Competing interests

All authors have no any conflict of interest

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Abstract

Bacterial infections in hospitalized patients with liver cirrhosis in Egypt increased during last decades. We target using safe probiotics treatments to overcome multidrug resistant bacterial infection. Out of twenty patients recruited, MDR bacteria were isolated from five patients. The results indicated that both the total viable bacterial counts and resistance of MDR bacteria to different antibiotics reduced significantly after *Lactobacillus acidophilus LB* treatment. 16S rRNA results indicated that the two strains were *E. coli* ABA8 and *K. pneumoniae* DSM-30104. This research concluded the importance of using probiotics to avoid bacterial secondary infection in patients with chronic hepatic disease.

Key words: chronic diseases- liver- cirrhosis – multidrug resistant bacteria- probiotics

Introduction

Chronic disease is defined as an illness that lasts at least one year and requires ongoing medical care as well as limited access to daily activities. Chronic diseases such as cancer, diabetes mellitus (DM), cardiovascular diseases (CVDs), and kidney disorders, can be infectious or non-infectious (Bazaid *et al.*, 2022). These chronic diseases are widespread throughout the world, while greater morbidity rates are associated with low-income and/or developing nations, including Egypt.

Cirrhosis of the liver is the most prevalent outcome of practically all chronic liver illnesses, accounting for about one million deaths worldwide each year. Bacterial infections affect more than 25% of all hospitalized patients with decompensated liver cirrhosis. In turn, infections are well-known precipitators of cirrhosis-related complications and acute chronic liver failure (Jalan et al., 2014). Several variables as changes in the gut microbiome could enhance

the chance of bacterial translocation which in turn contribute to people with liver cirrhosis' vulnerability to bacterial infection (Piano *et al*., 2019)**.**

Liver cirrhosis causes progressive immunological dysfunction resulting in a secondary bacterial infection which affect the cardiovascular response resulting in a fast hemodynamic collapse (Bunchorntavakul and Reddy, 2016)**.** So, to improve the prognosis of patients with liver cirrhosis, it is critical to identify potential risk factors for infections and to begin appropriate antibiotic treatment in patients who show signs of infection (Fernandez *et al*., 2019).

Multidrug- resistant bacteria (MDR) bacteria increased incredibly in last years (Shaaban and El-Sharif, 2001; Shaaban *et al*., 2021)**.** So, antibiotic selection must be guided by the local epidemiology of multidrug-resistant (MDR) bacteria. In this context, a recent global study projected the rate of MDR bacteria to be as high as 36% globally, less than 20% in the United States, and more than 70% in India(Labenz *et al*., 2020;Abdel-Aziz *et al*., 2021) .

Any alternation in the abundance or diversity of the gut microbial community can potentially affect microbial balance between the host and the gut microbial community (Kho and Lal, 2018). Studies have reported the proper effect of probiotics on gut microbial community balance so it can be a promising alternative to high dose antibiotics against a variety of gastric disorders (Lo *et al*., 2014)**.**

Therefore, this study aimed to evaluate the bacterial infections in hospitalized patients with liver cirrhosis and to analyze the antibiotics sensitivity and prevalence of MDR bacteria before and after patient treatment by probiotics.

Material and methods

Study design

This is a prospective cross-sectional study that was carried out at El-Askari Cairo Hospital, Egypt. This study included data from twenty individuals with chronic illnesses (hepatic cirrhosis and end stage liver disease). All participant information, including diagnosis, gender, isolated bacteria, and resistance profiles, was gathered. All participants gave informed consent and the experiments were performed according to Institutional Review Boards (IRBs) guidance.

Sample collection, isolation and counting of bacterial colonies

Fecal samples were collected from patients before and after treatment by probiotics according to the hospital policy on days 2,7,14. The samples were weighed and homogenized. Collected samples were serially diluted and plated on MacConkey (Oxoid Ltd., England) for analysis of Gram-negative bacteria then incubated overnight at 37 ºC. After isolation, Chrom agar medium (Oxoid Ltd., England) used for counting of the antibiotics resistant bacterial colonies using colony forming unit (CFU). Then, bacterial colonies were purified and preserved for further study (Penfornis and Pochampally, 2016).

Antibiotics sensitivity of isolated pathogenic bacteria

The antibiotic sensitivity testing (AST) and resistance pattern of bacterial pathogenic isolates were detected against 16 antibiotics using VITEK 2 next generation system (bioMérieux United States of America)(Ling and Liu , 2003)**.**

Growth curve and administration of probiotic bacteria

This test was done to determine the duration between each probiotics dose. Tubes containing 10 ml of MRS broth medium supplemented with 1% (w/v) carbohydrate were inoculated and incubated at 37 ◦C in shaking incubator at 50 rpm for 26 h. At this period, samples were collected every two hours and the absorbance was measured using a spectrophotometer at a wavelength of 580 nm. Probiotic (*Lactobacillus acidophilus LB* strain**)** sachets has been taken immediately by the patients at the first time and then followed by one sachet twice a day for two weeks. Samples were collected after treatment and antibiotic sensitivity were estimated.

DNA Barcoding and Phylogenetic Analysis

16S rRNA used for identification of the most antibiotics resistant isolates. A comparative analysis of sequences was performed using the CLUSTAL W multiple sequence alignment program, version 1.83 of MegAlign module of Lasergene DNA Star software Pairwise, which was designed by (Thompson *et al*., 1994) and Phylogenetic analyses were done using maximum likelihood, neighbor joining and maximum parsimony in MEGA6 (Tamura *et al*., 2013)**.**

Statistics

All biological experiments were done in triplicates. Statistics were done using the SPSS software. The results were expressed as mean values ± standard deviation (S.D.) and the significance between treated and non-treated groups was considered when the p-value is equal to or less than 0.05 between the compared treatments.

Results and Discussion

The different bacterial isolates were screened over twenty patients who suffered from liver diseases varied from ascites to end stage liver disease and HCV which caused liver cirrhosis and liver failure. All of patient were diabetics and suffered from hypertension. They finally were prepared for liver transplantation surgery**.** A microbial infection is a potentially fatal occurrence that can result in major complications such as sepsis and multiple organ dysfunction syndrome as well as mortality particularly in individuals with chronic diseases. (Fernandez *et al*., 2019)**.**

There are few studies on bacterial infections in multiple chronic disease patients at the same time to compare their overall prevalence and antibiotic resistance profiles (Shallcross and O'Brien, 2017). The current study aims to look at the prevalence of bacterial infections and the sensitivity profiles of causative strains in individuals with chronic liver disease before and after treatment with probiotics.

Isolation was performed on MacConkey agar before and after treatment with probiotics. The results indicated that out of twenty patients only five MDR isolates were obtained whose patients were further treated with probiotics and further studied. The most prominent isolates were identified biochemically using VITEK 2, four isolates were verified as *E. coli* whereas the remained one was verified as *K. pneumoniae.* So, patients with liver cirrhosis have an increased risk of infection by these pathogens(Righi, 2018;Lingiah and Pyrsopoulos, 2020)**.**

After that, as shown in table (1) and (2), the total MDR viable bacterial counts (CFU) were estimated on Chrom agar media. Almost all of the bacterial isolates were multi-drug resistant pathogenic isolates which verified using antibiotic sensitivity pattern using VITEK 2(Ling T, Liu Z, 2003). Also, many researchers reported that, there are high levels of antimicrobial drug resistance bacteria isolated from patients with chronic liver disease*(Fernandez et al*., 2019; Patel and Williams, 2020).

The results indicated that bacterial count of samples from patients treated with probiotics were reduced significantly after treatment has been taken when compared with that of before treatment. The results in fig. (2) revealed that the logarithmic phase for probiotic bacteria ended after 12 hours Table (2) showed the total mean and standard deviation (S.D.) of bacterial colony forming unit for all patients before and after treatment with probiotic, it also showed the Z value and significant for the readings of the whole group. There were statistically significant differences between the mean readings of the research samples before and after using probiotic as the level of significance $\alpha \leq 0.05$, where Z value was 2.92. Also, Jeong et al**.** (Jeong *et al*., 2022) recommend using *Lactobacillus* probiotics in liver disease treatment as it is effect on decreasing CFU of pathogenic microbes .

As shown in table (3), the sensitivity of different *E. coli* and *Klebsiella pneumoniae* strains isolated from different patients before undergoes probiotic treatment this shows that the 1st *E. coli* strain was sensitive to only Ticarcillin/ Clavulanic acid otherwise it was sensitive to all. The 2nd *E. coli strain* was resistant to all antibiotics except Moxifloxacin, Tetracycline and Tigecycline and Intermediate to Meropenem. The 3rd *E. coli* strain was sensitive to Piperacillin, Moxifloxacin, Minocycline, Tetracycline, Tigecycline, Chloramphenicol and Trimethoprim otherwise it was intermediate to Ticarcillin/ Clavulanic acid. The 4th *E. coli* strain was sensitive to Piperacillin, Cefuroxime,

Levofloxacin, Minocycline, Tetracycline, Chloramphenicol and Trimethoprim while it was intermediate to Ticarcillin/ Clavulanic acid and resistant to the rest of antibiotics.

Table (4) showed the sensitivity of different *E. coli* and *Klebsiella pneumoniae* strains isolated from different patients after probiotic treatment. This shows that the 1st *E. coli* strain was sensitive to all antibiotics. The 2nd *E. coli* strain showed intermediate sensitivity to both Ticarcillin/Clavulanic Acid and Tigecycline while it was sensitive to the another of antibiotics. The 3rd *E. coli* strain showed sensitivity to all antibiotics except Tigecycline which showed intermediate effect. The 4th *E. coli* strain showed sensitivity to all antibiotics except Ticarcillin/Clavulanic Acid and Cefixime as it showed intermediate sensitivity. The *Klebsiella pneumoniae* strain was sensitive to all antibiotics except it was resistant to Piperacillin only. This may be attributed to the effect of probiotics on immune system and biofilm formation by resistant bacteria (Jeong *et al*., 2022) and the modulation of intestinal microflora through the use of probiotics (Lo *et al*., 2014).

Biochemical identification of bacterial isolates was done using VITEK 2, the results indicated that the five prominent isolates from patients were *E. coli* and *K. pneumoniae***.** For more confirmation, 16S rRNA used for identification of the antibiotic resistant isolates. The results confirmed that, antibiotics resistant isolates analyzed showed high similarity with *E. coli* ABA8 (figure 2). And also high similarity with *K. pneumoniae DSM*- 30104 (figure 3). Also, *Fernández et al.* (Fernandez *et al*., 2019)reported the prevalence of *K. pneumoniae* in patients with decompensated cirrhosis and acute chronic liver failure in Europe

Conclusion

Antibiotics resistant bacteria were predominant in tested patients with chronic liver diseases. Treatment of these patients with probiotics significantly reduce viable MDR bacterial count. Also, probiotics significantly decrease bacterial antibiotics resistance as detected by antibiotic sensitivity pattern of isolated bacteria before and after treatment. Finally, further studies were needed for progressed application of probiotics for liver transplantation patients.

Authors contributions

Mohamed T. Shaaban, exeperiments design, interpretation of data and manuscript revision.

Gehan M. Fahmy, manuscript revision and interpretation of data.

Hussein S. Salama, statistical analysis, interpretation of data and manuscript revesion.

Esraa Hafez, exeperimental work and writing manuscript

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Tables

Table (1): The bacterial count of isolates from liver transplantation patients before and after treatment with probiotics **(the data were represented as mean value ± standard deviation).**

Patient No.	Average colony counts before treatment (CFU)	Average colony counts after treatment (CFU)
No. 1	$1.3*10^7 \pm 1*10^6$	$5.0*10^2 \pm 7.5*10^2$
No. 2	$1.23*10^6 \pm 5.7*10^4$	$1*10^3 \pm 1*10^3$
No. 3	$1.73*10^7 \pm 3*10^6$	$6.0*10^3 \pm 2*10^3$
No. 4	$1.97*10^8 \pm 1.5*10^7$	$5.23*10^3 \pm 6.8*10^2$
No. 5	$1.77*10^8 \pm 2.08*10^6$	$7.0*10^2 \pm 2.6*10^2$

Table (2): A comparative analysis between the bacterial count before and after probiotic treatment has been taken.

Treatment with probiotics	Mean	Standard deviation (S.D.)	Z value	Significant
Before	**$8.093*10^7$**	**$9.696*10^6$**	**2.92**	**0.01**
After	**$0.000257*10^7$**	**$0.00028*10^6$**		

Figures

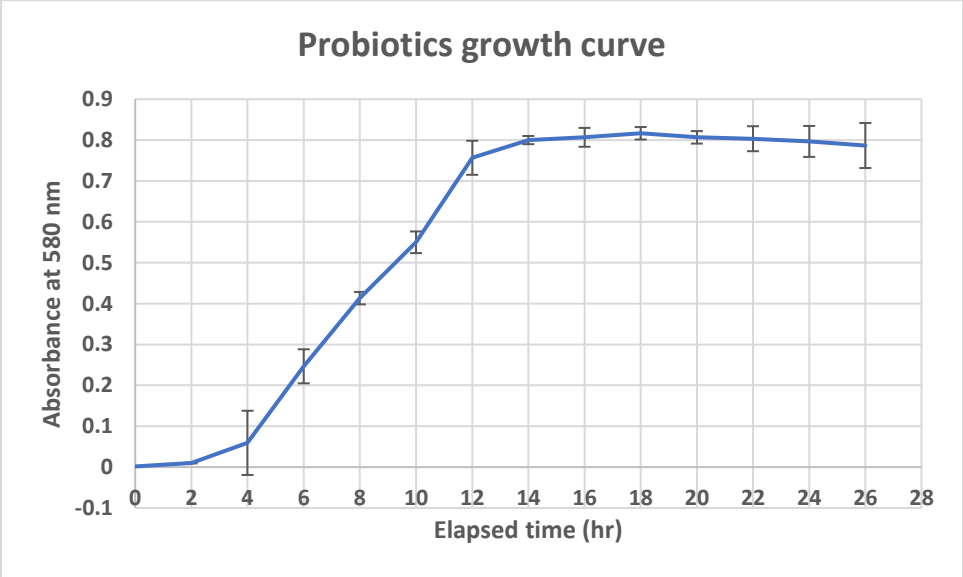

Fig.1. Growth curve of probiotic bacteria.

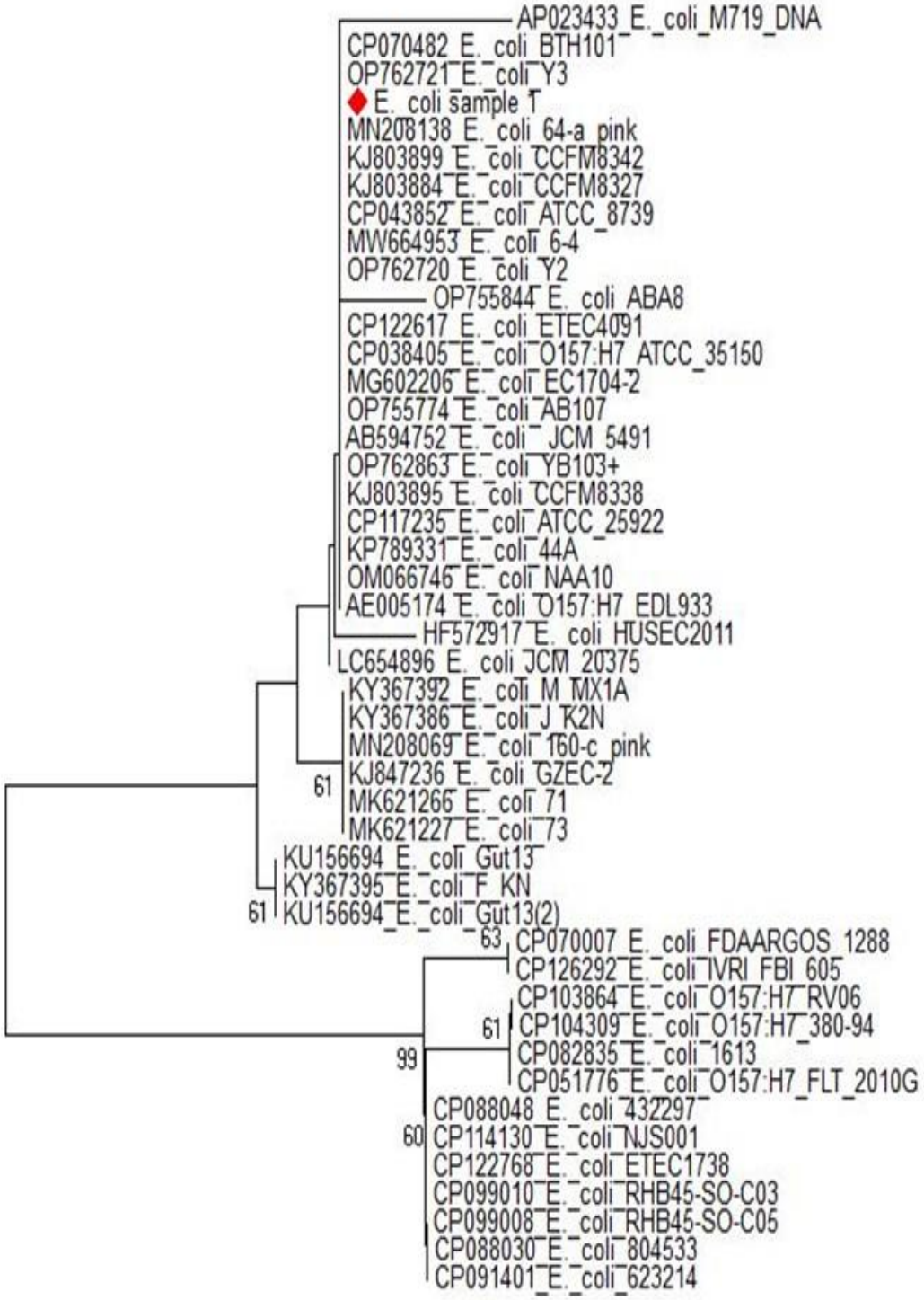

Fig. 2. phylogenetic tree of the most antibiotic resistant *E. coli* strain

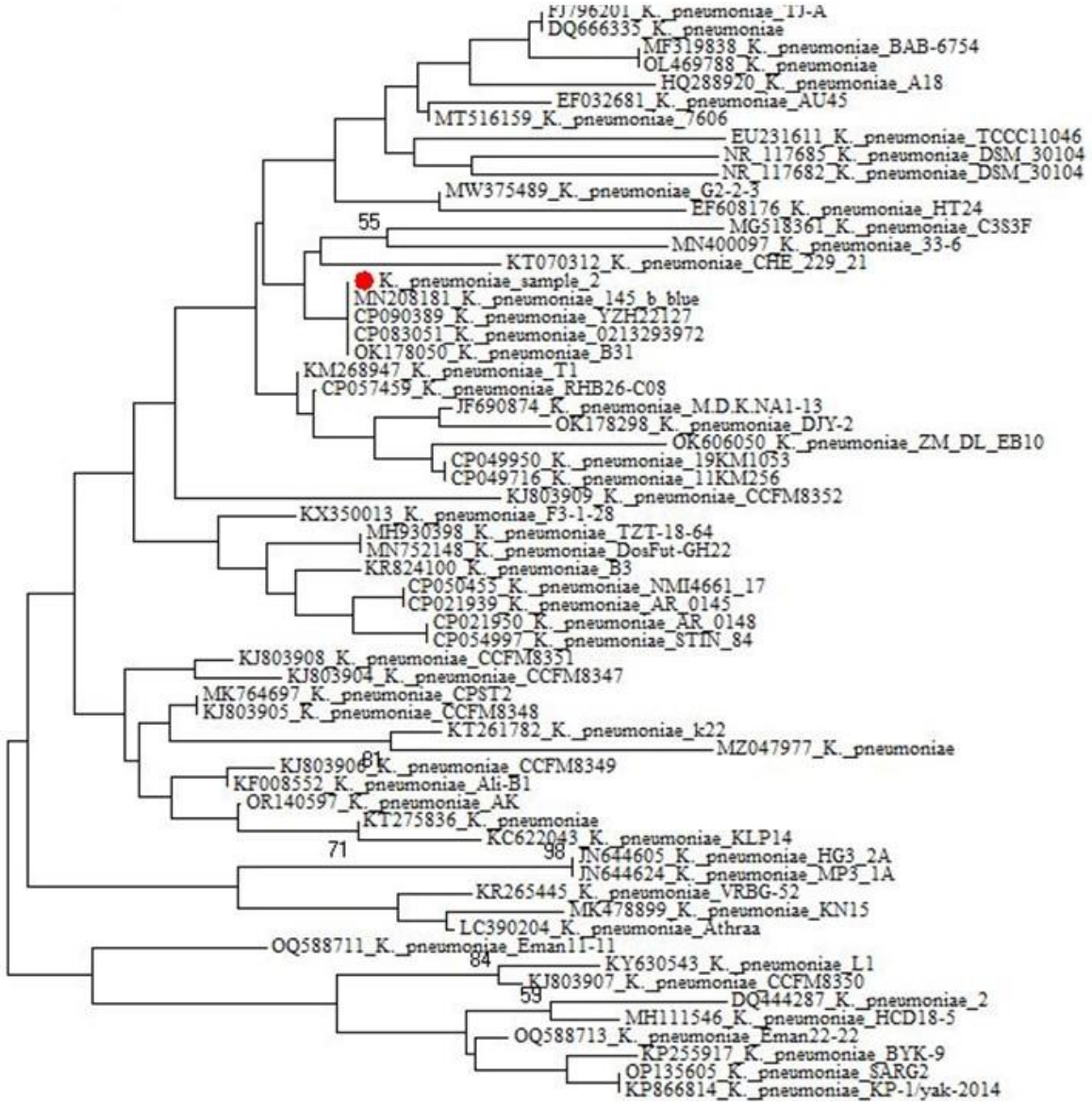

Fig. 3. phylogenetic tree of the most antibiotic resistant *K. pneumoniae* strain.

Table 3: The antibiotics sensitivity of the different bacterial fecal isolates before treatment. (ATM; Aztreonam, FEP; Cefepime, CFM; Cefixime, CRO; Ceftriaxone, CXM; Cefuroxime Axetil, CFX; Cefuroxime, CHL; Chloramphenicol, LVX; Levofloxacin, MEM; Meropenem, MIN; Minocycline, MXF; Moxifloxacin, PIP; Piperacillin, TET; Tetracycline, TI; Ticarcillin/Clavulanic Acid, TGC; Tigecycline, TMP; Trimethoprim).

***Strain/* Antibiotics**	TIM	PIP	CFX	CXM	CFM	CRO	FEP	ATM	MEM	LVX	MXF	MIN	TET	TGC	CHL	TMP
E.coli (1)	S	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
E.coli (2)	R	R	R	R	R	R	R	R	I	R	S	R	S	S	R	R
E.coli (3)	I	S	R	R	R	R	R	R	R	R	S	S	S	S	S	S
E.coli (4)	I	S	S	R	R	R	R	R	R	S	R	S	S	R	S	S
K. pneumoniae	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R

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Strain/ Antibiotics	TIM	PIP	CFX	CXM	CFM	CRO	FEP	ATM	MEM	LVX	MXF	MIN	TET	TGC	CHL	TMP
***E.coli* (1)**	S	S	S	S	S	S	S	S	S	S	S	S	S	I	S	S
***E.coli* (2)**	I	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
***E.coli* (3)**	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
***E.coli* (4)**	I	S	S	S	I	S	S	S	S	S	S	S	S	I	S	S
K. pneumoniae	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S

Table 4: The antibiotics sensitivity pattern of the different bacterial fecal isolates after treatment with probiotics. (ATM; Aztreonam, FEP; Cefepime, CFM; Cefixime, CRO; Ceftriaxone, CXM; Cefuroxime Axetil, CFX; Cefuroxime, CHL; Chloramphenicol, LVX; Levofloxacin, MEM; Meropenem, MIN; Minocycline, MXF; Moxifloxacin, PIP; Piperacillin, TET; Tetracycline, TI; Ticarcillin/Clavulanic Acid, TGC; Tigecycline, TMP; Trimethoprim).