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Isolate and Molecular Study of Pathogenic Bacteria and Study the Activity of Silver Nanoparticales and *Lactobacillus acidophillus* on it.

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Abstract

Diarrhea is the passage of remarkably loose or watery stools, generally more than three times within 24 hour. Hence this training marked to identify the causative agents in children by using phenotypic and molecular methods.

A total of 200 stool samples were collected from patients suffering from diarrhea during a period from August 2017 to Desember2017m , in a Woman and children BABIL Hospital in Hila province,found 127 samples(63.5%) were positive for bacterial culture while, 73 samples (36.5 %) showed negative results.The result of the biochemical test revealed that 40/127(31%)of bacterial isolates were *E.coli*,10/127 (7.8%)of isolates were *Proteus* ssp ,and 20/127(15.7%) isolate were *Klebsilla* ssp,26.in /127(20%)isolate were and *Enterobacter* ssp, *Shigella* identification in10/127 (7.8%),*Salmonella* in12/127(8.6%),*Pentoea* in10/127(7.8) isolate. The Molecular technique used to detect the presence of genes that responsible for *E.coli* resistant to antibiotics by using specific primers are *(aac,tet,ctxm,parc*) the results showed that 40 of *E.coli* isolate carried these genes ,the amplification of *aac* gene was observed in (1-4,6,8,9,10)sample number ,*ctxm* was observed in (1-13*),tet* was observed in (1-11),*parc* was in (1-4,7,8,9,10) sample number . In this study ,it has been detect the antimicrobial effect of silver nanoparticles and *lactobacillus acidophillus* probiotic on the bacterial isolate,the result showed that *Proteus* ssp isolate,were the highly effected while, the antibiofilm activity result showed that *Pentoea* and *Shegilla* ssp were the highly effected.

Keywords : Diarrhea , *E.coli. aac, tet, ctxm ,parc*, Silvernanoparticals , *lactobacillus acidophillus*

INTRODUCTION

The Diarrhea is a sudden increase in incidence and looseness of bowel energy. It is hard to describe diarrhea exactly, since there is greatindividual difference in normal stool frequency, Pointers of diarrhea comprise a unexpected increase in number of stool occurrence, a reduction in stool constancy, more watery stool and a tendency for stool to be green[(1)]. There is no agreement on when diarrhea becomes chronic, but the shortest duration after which diarrhea may be termed chronic is probably 4 weeks[(2)].Enteric viruses are the main etiologic managers of acute gastroenteritis amongst infants and young children worldwide,the main detached ofthe study was to determine the role of enteric viruses in acute diarrhea in the country [(3)].

Virulence factor denote to the properties that allow a microorganism to institute itself and duplicate on or within exact host species, and that improve the microbe's potential to cause disease[(4)]The resistant of bacteria to the antibiotic increasing amongst the bacterial isolates from patients in both outpatient and in patients ,which are considered as a focus infection .this resistance can be developed by modification or by plasmid of resistance genes from other microorganisms[(5)] .

The study was amid to detect the most important pathogenesis of diarrhea and determine the role of common gens responsible for antibiotics resistance by the following objectives.

1. Isolation and certification of bacteria that cause diarrhea in children under 6 year by traditional methods and approve by ViteK-2.
2. Detection the genes that resist to antibiotic by PCR technique.

METHODS

Patients and specimens collection

A total of 200 stool specimens were collected from infants and children present at WOMAN AND CHILDREN BABLE Hospital, who were suffering severe diarrhea. The specimens were collected from patients below six years of age and from both sexes , and put in sterile plastic containers of sufficient size and within a tight fitting leak-proof lid were used. The specimens were delivered to the laboratory, and treated directly or as soon as possible, but often not more than two hours after collection

Isolation and Identification of Bacterial Isolates

Bacteria diagnosed depending on the morphological characteristics on the culture media and confirm the diagnosis by using biochemical test.

Polymerase chain reaction (PCR) assay

The DNA extracted from samples were tested with PCR for DNA with the general primer-***aac* , *ctxm* ,*tet*** , and ***Parc***, all samples were analyzed for the presence of this genes and negative controls (water) were used in each batch of PCR and the negative controls were added after all the samples were added to the PCR tubes. None of the negative controls were positive. Amplified PCR products were analyzed by electrophoresis in a 1.5% agarose gel containing TAE buffer and ethidium bromide (Sigma, Castle Hill, Australia). PCRamplicons were size determined under UV light using the GelDoc software (Bio-Rad, Sydney, Australia). [(6)]

Effect of silver nanopartical and lactobacillus acidophillus on bacterial isolate

The microorganism was inoculated on brain heart infusion broth ,at 37c for 24hours.(first activation). And used the same method for (second activation) also used antimicrobial activity of silver nanopartical and lactobacillus acidophillus on isolates under study. [(7)].

Statistical Analysis

Data were analyzed using online statistical software (VassarStats, Vassar College, Poughkeepsie,NY) and tested using the Chi-square test.

RESULT AND DISCUSSION

The present study included 200 clinical stool specimen, 127/200(63.5%) specimens were positive culture (bacterial causes),and the other 73/200(36.5%) isolates were considered negative results.The biochemical tests which appeared that of isolates were40/127 (31%) *E.coli*, 10/127 (7.87%) isolates were *Proteusssp* ,20/127(15.7 %) isolates were *Klebsella* ssp,26/127 (20%) *were Enterobacter*ssp , *Shigella* were in10/127 (7.8%),11/127 (8.6%) *of ssp. Salmonella* and *Pentoea* appeared in10/127 (7.8%)isolates as show in table (1)

Detection of *aac* gene in *E.coli*

Polymerase chain reaction technique of *E.coli* clinical isolates revealed that *aac* gene were gave positive result for this gene with product size 490 bp

This study is similar to the study by [8] who detected *E.coli* isolates from different sources .

Detection of *ctxm* gene in *E.coli*

Polymerase chain reaction technique of *E.coli* isolates revealed that *ctxm* gene were gave positive result for this gene with product size (543bp) as showen in figure (2).

The result of our study is like to the study of [9] who found that bla_{CTX-M} β-lactamase was the most prevalent among the ESBL producing G-ve isolates; followed by bla_{TEM} β-lactamases were the less . In India [10], reported that bla_{CTX-M} β-lactamases were more common enzymes than bla_{TEM} in clinical negative isolates [11]. PCR is considered an efficient method for ESBL detection, because it is faster than phenotypic detection method [12], in addition to detect the presence of poorly or non-expressed (silent) genes, that is difficult to determine by phenotype methods; PCR may also be used to directly test samples as an early predictor of infection [13].

Table 1 - The positive result of the bacterial isolate from diarrheal children

Bacterial species	No	Percentage
E.coli	40	31%
Proteus ssp	10	7.87%
Klebsilla ssp	20	15.7%
Enterobacter ssp	26	20%
Shigella ssp	10	7.8%
Salmonella ssp	11	8.6 %
Pentoea ssp	10	7.8%

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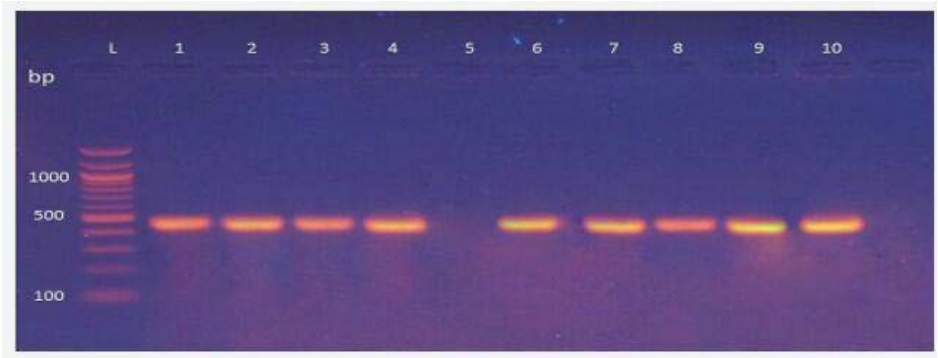
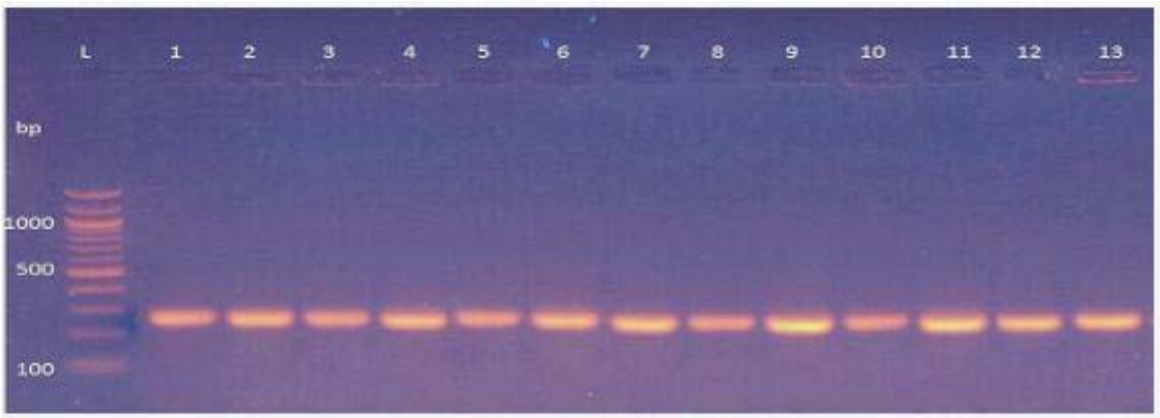
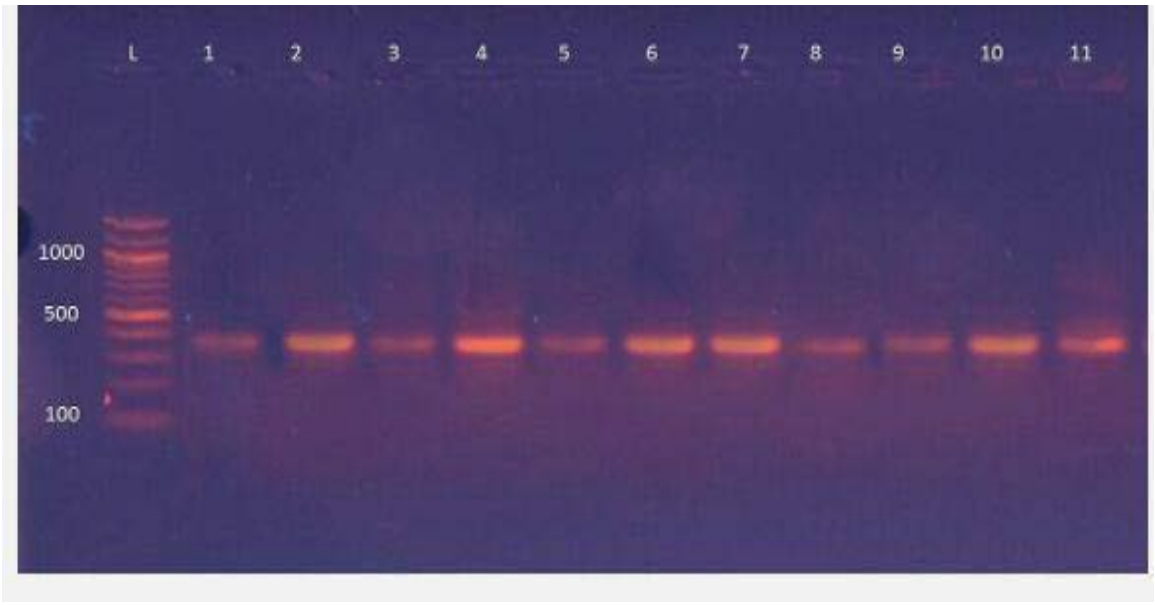


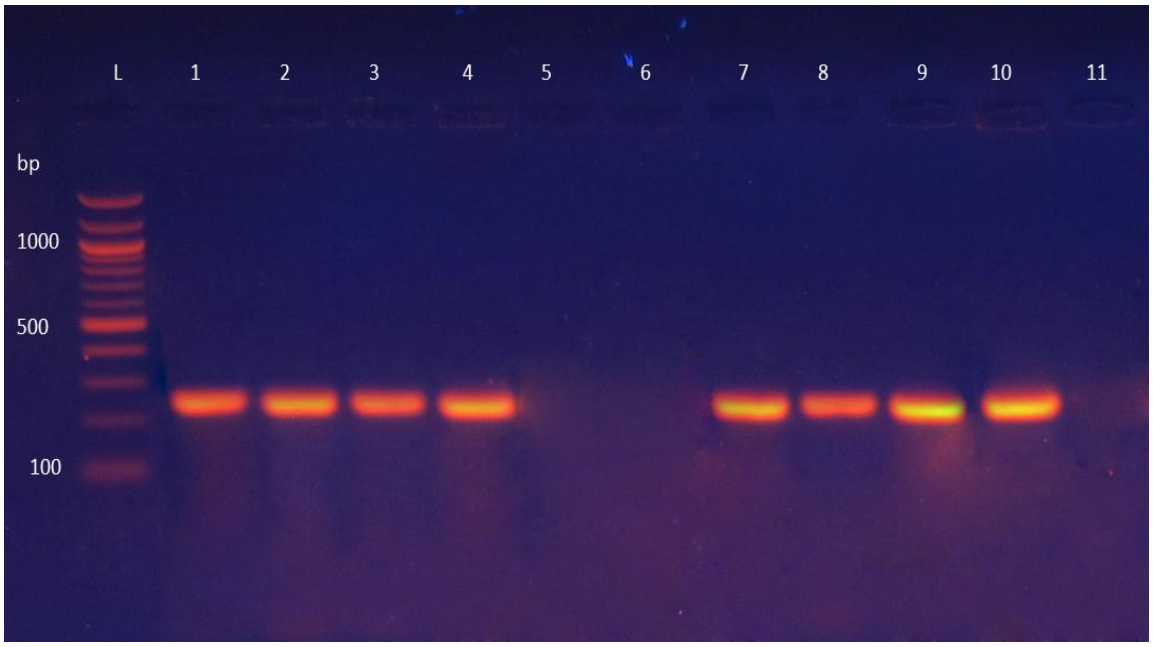
Figure(1)Ethidium bromide stainagarose gel for PCR amplification products of *E.coli* isolates that amplified with *aac* gene primer with product size 490bp.(1.5%agarose gel ,75 v,1.20 hours). lane(L),DNA molecular size marker(100_bp ladder),lanes(1_4,6,7,8,9,10)show positive result with *aac* gene.



Figure(2):Ethidium bromide stain agarose gel of PCR amplification products of *E.coli* isolates that amplified with *ctxm*gene primer with product size 543bp.(1.5%agarose gel ,75 v,1.20 hours).lane(L),DNA molecular size marker(100_bp ladder),lanes(1-13)show positive result with *ctxm*gene.



Figure(3):Ethidium bromide stain agarose gel of PCR amplification products of *E.coli* isolates that amplified with *tet*gene primer with product size 377bp.(1.5%agarose gel ,75 v,1.20 hours).lane(L),DNA molecular size marker(100_bp ladder),lanes(1_11)show positive result with *tet*gene.



Figure(4):Ethidium bromide stain agarose gel of PCR amplification products of *E.coli* isolates that amplified with *parc*gene primer with product size 264bp.(1.5%agarose gel ,75 v,1.20 hours).lane(L),DNA molecular size marker(100_bp ladder),lanes2(1-4,7,8,9,10)show positive result with*Parc*gene.

Detection of *tet* gene in *E.coli*

The gene *tet* of *E.coli* appear as positive result by pcr technique with product size (337bp) as shown in figure (3).

Tetracycline is clinically known as broad spectrum antibiotic and is effective against many Gram-positive and -negative bacteria. Tetracycline mostly act by stopping the binding of tRNA to the A site of the 30S rRNA, causing misreading of the mRNA code or inhibition of the initiation step of protein synthesis [(14)].

Detection of *Parc* gene in *E.coli*

Parc gene of *E.coli* give a positive result by PCR Technique with size(264bp).

The first is the targeting mutations in the quinolone resistance-determining region (QRDR) of two *gyrA* and *gyrB* genes, which encode for the subunits of DNA gyrase, and/or in *parC* and/or *parE* subunit of topoisomerase IV [(15)].

Distribution of genes according to *E.coli* isolate

Table(2): Distribution of four genes according to *E.coli* isolate

E.coli	*AAC*	*TET*	*CTXM*	*PARC*
1	+	+	+	+
2	+	+	+	+
3	+	+	+	+
4	+	+	+	+
5	_	+	+	_
6	+	+	+	_
7	+	+	+	+
8	+	+	+	+
9	+	+	+	+
10	+	+	+	+
11	_	+	+	_
12	_	_	+	_
13	_	_	+	_
14	_	_	_	_
15	_	_	_	_
16	_	_	_	_
17	_	_	_	_
18	_	_	_	_
19	_	_	_	_
20	_	_	_	_
21	_	_	_	_
22	_	_	_	_
23	_	_	_	_
24	_	_	_	_
25	_	_	_	_
26	_	_	_	_
27	_	_	_	_
28	_	_	_	_
29	_	_	_	_
30	_	_	_	_
31	_	_	_	_
32	_	_	_	_
33	_	_	_	_
35	_	_	_	_
36	_	_	_	_
37	_	_	_	_
38	_	_	_	_
39	_	_	_	_
40	_	_	_	_

Pathogenic bacteria can gain resistance to antibiotics through horizontal gene transfer of naturally occurring antibiotic resistance genes (ARGs) native to other microbes or de novo mutations under environmental pressure [16].

The Plasmid-mediated quinolone resistance mechanism captures a variant of aminoglycoside acetyl-transferase *aac(6')-Ib-cr* gene, which can diminish a fluoroquinolone activity by adding an acetyl group to this antimicrobial agent [17].CTX-M genes are the recent family of plasmid-mediated ESBLs; [18] .The success of the CTX-Ms over the classical ESBL-enzymes is linked to the way by which CTX-M enzymes were spread [19].Resistance to quinolone has been classified into two mechanisms. The first is the targeting mutations in the quinolone resistance-determining region (QRDR) of two *gyrA* and *gyrB* genes, which encode for the subunits of DNA gyrase, and/or in *parC* and/or *parE* subunit of topoisomerase IV [20].

Antimicrobial activity of silver nanoparticales and *Lactobacillus acidophillus* on the bacterial isolate

The effect of silver nanoparticales and *L.acidophillus*revealed that 25of *E.coli* isolates are effected by silver nanoparticales and 15 isolates are effected by*L.acidophillus,*10 of *klebsilla* isolatesare effected by silver nanoparticales and 10 are effected by *L.acidophillus*, 9 of *salmonella isolates*are effected by silver nanoparticales and 2 are effected by *L.acidophillus*, 6 of *shigella* isolatesare effected by silver nanoparticales and 4 are effected by *L.acidophillus*,8 isolates of *Proteus* isolate are effected by silver nanoparticales and 2 are effected by*L.acidophillus*,6 of *pentoea* isolate are effected by silver nanoparticales and4 are effected by *L.acidophillus* as shown in table (3).

Table (3) Antimicrobial activity of silver nanoparticles and *L.acidophillus* on bacterial isolate

Type of bacteria	Silver	L.acidophillus
E.coli	12	10
Klepsilla	8	10
Salmonella	10	10
Shigella	8	7
Proteus	12	11
Enterobacter	10	8
Pentoea	6	4

CONCLUSION

In end, our outcomes tested the attendance of the pathogenic bacteria showed the most common pathogens are *E.coli* then *proteus* and the less *Pentoea*. The Molecular technique appeared four genes *(aac,tet,ctxm,parc*) observed in more one isolates of *E.coli* and the (aac gene) Is the most common in isolates than others . *Proteus* ssp ,were the highly effected by silver nanopartical and *lactobacillus acidophillus*.

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