Variation of 16SrRNA gene for Some Nosocomial Bacteria Isolated from Ramadi Teaching Hospital for Women and Children

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Abstract

The aim of this studsy is to diagnose bacterial strains in hospitals by the 16SrRNA gene and to know the variation of the nucleotide sequence. The study of bacteria in hospitals is of great importance to identify the extent of change in these species and their resistance to antibiotics. This indicates that these bacteria have the ability to change or obtain new variants to be able to resist antibiotics. In this study, samples were taken from different locations of the Ramadi Teaching hospital for women and children and were cultured on different media. They were also stained and examined under light microscopy and some biochemical tests were conducted. Pure colonies were obtained after re-culture and then extracted the DNA from the 16 isolated samples and measuring its concentration. Using the specific primer for 16SrRNA genes, the samples were amplified by a PCR and the size was shown to be 1500 bp. The unknown bacteria were sent to the sequence. Some samples showed variations in comparison with the data presented by NCBI. This is evidence of some variations shown by bacteria to overcome antibiotics and increase their severity and spread in hospitals, whether in kitchens, emergency or preterm infants, as well as laboratory staff and lab equipment.

Keywords: *16SrRNA gene, Nosocomial infections, Transition, Transversion, Transduction*

Introduction

Nosocomial infections are as well-known as hospital-acquired/associated infections and common problem that increases the duration of stay hospital, hospital cost often affects patients' quality of life, survival, and response to therapy [1,2]. It is an infection acquired by the patient length a hospital stays. Lately, a modern term is used, "healthcare-related infections" this term used for the type of infections give rise to by a lengthy hospital stays, and it accounts for a major risk factor for serious health issues leading to doom [3]. Patients' food is prepared in the hospital kitchen and is then distributed throughout the wards also the worker in hospital kitchen [4]. Patients are more prone to infections in comparison to healthy individuals [5]. Even length of hospitalization can be influenced by factors such as undernourishment, food poisonings, and hospital infections [6]. Invasive devices such as catheters and ventilators employed in modern health care are associated to these infections and factors such as critical origins, not enough cooking, keeping at improper temperature, and lack of personal hygiene are important factors in the incidence of food-borne diseases [7]. There are statistics showing that out of every 100 patients receiving treatment in hospitals, seven in developed countries and ten in developing countries can acquire one of them related to healthcare infections [8]. Therefore, spread or outbreak of microorganisms in hospitals can result in more serious outcomes for patients [9]. So, one of the principal goals of hospitals is the provision of healthy food for patients who are susceptible to infections and complications [10]. Nosocomial infections appeared prior to the buildup of hospitals and became a health problem through the prodigious antibiotic era. Because of these infections, not only the costs until the use of antibiotics increased with extended hospitalization and lead to increase the resistance against the antibiotic. Studies behave

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in various regions of the world show that in North America and Europe 5%-10% of all hospitalizations result in nosocomial infections, while South America, Africa, and Asia exhibit exceed 40% hospitalizations with these infections [11]. The most common types of nosocomial infections include bloodstream infections, catheter-associated urinary tract infections, surgical site infections which lead to happen the gangrene, ventilator-associated pneumonia and lastly the food poisoning. [12]. The aim of this paper is detection of variation for some nosocomial bacteria isolated from Ramadi Teaching Hospital for Women and Children.

Materials and Methods

Sample Collection and Bacterial Identification

In the period from May 2018 to July 2018 collected forty sample from the food utensils, work surfaces and their contact surfaces as well as hands, work clothes, cutting boards and submitting food place. Total of 40 samples were collected aseptically in sterile disposable containers. Specimens were labeled and transported to the microbiology laboratory. The smears were streaked on MacConkey, Nutrient and Blood agar and all dishes were incubated at 37°C for 18 to 24 hours. Whole isolates were identified utilizing for biochemical test use Simone citrate agar, Kligler iron agar, Trypton broth, Catalase reagent and IMVIC test. After biochemical tests sixteen sample sent to diagnosis by the molecular method for detection 16S rRNA gene.

DNA Extraction

Genomic DNA was extracted from the samples was isolated from hospital using (DNA mini kit that was supplied by Promega DNA extraction kit, USA) according to manufacturer's instructions.

Quantitation of DNA

Quantus florometer was used to detect the concentration of extracted DNA in order to detect the goodness of samples for downstream applications. For 1 µl of DNA, 199 µl of diluted Quanty Flour Dye was mixed. After 5min incubation at room temperature, DNA concentration values were detected.

PCR Amplification:

The 16SrRNA gene was amplified using primer Forward 27F (5`-AGAGTTTGATCCTGGCTCAG-3`) and primer Reverse 149 2R (5`-TACGGTTACCTTGTTACGACTT-3`) [13]. The PCR amplification is performed in a total volume of 25µl containing 2 µl DNA, 12.5 µl Master Mix PCR (intron, Korea), 1µl of each primer 10 pmol and then nuclease-free water is added into a tube to a total volume from 25µl.

The Polymerase Chain Reaction program conditions were as follows: initial denaturation at five minutes at 95°C followed by thirty cycles of denaturation 95°C for 30 seconds, annealing at 60°C for 30-minute, extension at 72°C for one minute and the last extension of 72°C for 7 minutes. The PCR products were separated on 1% agarose gel. The gel is left to run for 90min with a 70volt/65 Am current. After PCR amplification, agarose gel electrophoresis was adopted to confirm the presence of amplification. PCR was completely dependable on the extracted DNA criteria.

Sequencing:

The sequencing of 16srRNA gene was performed at Macrogen Inc. using their ABI 3730xl genetic analyzer. Homology search was conducted using BLAST program which is available at the National Center Biotechnology Information (NCBI) online at http://www.ncbi.nlm.nih.gov and Bio Edit program.

Results and Discussion

Samples were isolated from various locations of Ramadi Educational Hospital for women and children (kitchen, preterm, emergency, lab, baby incubator, and some kitchen staff). After the isolation process, samples were taken to the laboratory and cultured on different media for the initial isolation and then cultured on enrich and differential media such as the blood agar medium, MacConkey agar, and the mannitol salt agar. It was observed that there was a variation in the colonies through the morphology and then was stain and examined under the optical microscope were some colonies appeared negative and some positive between the bacillus and coccus. The results of the biochemical tests showed variation between samples when we used the IMVIC test.

DAN Extraction

All sixteen sample activated on blood agar and extracted the DNA by Promega kit and measured the concentration by Quantus Florometer show table 1.

Table 1: DNA Concentration (ng/µl)

Sample	Concentration	Sample	Concentration
01	20	09	20
02	30	10	20
03	33	11	22
04	40	12	23
05	25	13	40
06	20	14	25
07	25	15	23
08	30	16	22

PCR Amplification

The electrophoresis results showed that the size band was 1500 bp using the ladder 1500bp after stain by Ethidium bromide in seen as fig. 1.

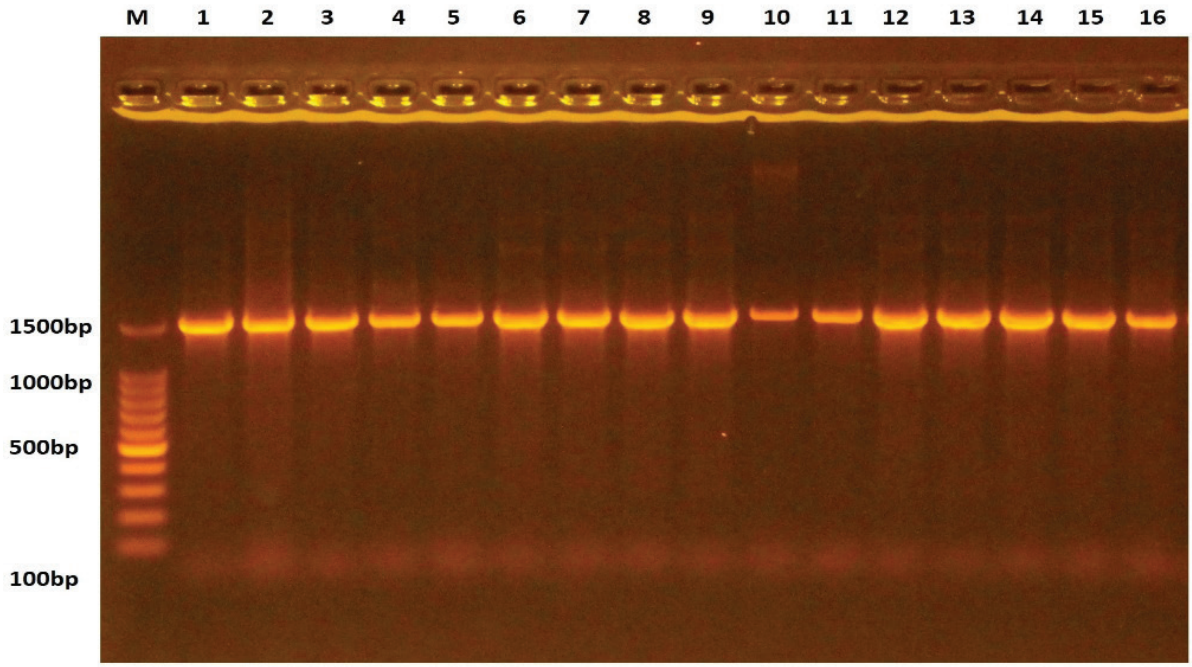

Fig.1: Results of the presence of 16SrRNA gene of Unknown bacterial species were fractionated on 1% agarose gel electrophoresis stained with Eth. bro.

Sequence Alignment of 16SrRNA Gene

The results of sixteen samples after send to sequence showed variation between the samples [14], the first sample *Pantoea calida* isolated from the lab, kitchen and emergency showed three variations all the variation (Transition) and the Second sample *Enterobacter hormaechei* isolated from lab, kitchen, preterm and chamber 1 showed two variation (Transition) table 2. While the other sample isolated from kitchen and lab *Pseudomonas putida* also show two variations (Insertion) and the *Kocuria* sp. isolated from kitchen and emergence and showed one variation (Transversion) table 3. Tenth sample *Micrococcus luteus* from all location in the hospital and showed one variation (Transversion). Also, the eleventh sample *Rothia* sp. gives the one variation (Transition) isolated from all location table 4. *Staphylococcus pasteuri* was prevalent in the all location and shown one variation (Transition). The fifteenth was *Staphylococcus saprophyticus* isolated from emergence, lab and kitchen shown one variation (Transition). Lastly, the *Lelliottia amnigena* was isolated from the clothes kitchen staff and show three variations first (Insertion) and two (Transition) table 5.

The samples 5,6,7,8,9,12 and 14 *Rothia* sp., *Planococcus* sp., *Lysinibacillus* sp., *Staphylococcus haemolyticus*,*Staphylococcuspasteuri*,*[Brevibacterium] frigoritolerans* and *Bacillus aryabhattai* subsequently don't show any variation in sequence.

Submission of Local Iraqi Isolates in NCBI

The16SrRNA gene was registered after the correspondence of NCBI and obtain accession number for the 1 and 16 sample. https://www.ncbi.nlm.nih.gov/nuccore/MN209789.1/ and https://www.ncbi.nlm.nih.gov/nuccore/MN215912.

Table 2: Represent Type of Polymorphism of 16srRNA Gene from *Pantoea calida* and *Enterobacter hormaechei*

No.	Type of substitution	Location	Nucleotide	Seq. ID
Isolate1	Transition	38	T>C	LC192167.1
	Transition	798	C>T	
	Transition	806	G>A	
Isolate 2	Transition	402	A>G	CP017180.1
	Transition	1081	T> C	

Table 3: Represent Type of Polymorphism of 16srRNA Gene from *Pseudomonas putida* and *Kocuria* sp.

No.	Type of substitution	Location	Nucleotide	Seq. ID
Isolate3	Insertion	52	- > A	AY395005.1
	Insertion	1402	-> T	
Isolate 4	Transversion	924	C>A	KX417302.1

Table 4: Represent Type of Polymorphism of 16srRNA Gene from *Micrococcus luteus* and *Rothia* sp.

No.	Type of substitution	Location	Nucleotide	Seq. ID
Isolate10	Transversion	1064	A>C	MH489048.1
Isolate 11	Transition	1383	G > A	KU884343.1

Table 5: Represent Type of Polymorphism of 16srRNA Gene from *Staphylococcus pasteuri*, *Staphylococcus saprophyticus* and *Lelliottia amnigena*

No.	Type of substitution	Location	Nucleotide	Seq. ID
Isolate13	Transition	456	T>C	MN004821.1
Isolate15	Transition	230	G > A	MK205161.1
Isolate16	Insertion	9	-> A	KM114915.1
	Transition	1192	A>G	
	Transition	1241	T>C	

Conclusion

We conclude from this research that the infection of patients to hospital bacteria is a serious risk to the patient because it possesses resistance to most antibiotics by acquiring methods through which to change the sensitivity of antibiotics of the important methods conjugation and transduction. Sequencing results showed that there were variations in the nucleotide sequence of some bacterial isolates isolated from the hospital compared to NCBI results.

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Conflict of Interest: Non

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