Genetic study of *Staphylococcus Aureus* Isolates from Different Environmental Source

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Abstract

Background: Bacteria *Staphylococcus aureus* are from nurses and serious task because of the ability to cause various types of injuries at different locations of the body, and that pathogenesis has nothing to do with their ability to produce many virulence factors.
Objective: To detection of virulence genes for *Staphylococcus aureus* isolates from different environmental source .
Patients and Methods: The study involves isolation and ldentifieation of 60 bacterial isolate ,10 of them was identified as Staphylococcus aureus ,which is collected different environments (soil ,water) . This study focused on the pathogenic bacteria S.aureus which was isolated from the natural sources (soil ,water) and was compared with S.aureus isolated from clinical samples isolates obtained from different clinical cases from Baquba General Teaching Hospital.
Results: This study includes 10 samples of *Staphylococcus aureus* that isolated from soil and water, 10 samples of *Staphylococcus aureus* that isolated from hospital, identification it and detection of resistant genes by used Polymerase Chain Reaction technique. 4 isolates were identified genetical by 16srRNA gene and detection of resistant genesfemA, nucby used Polymerase Chain Reaction technique. The results of the genetic identification of the 16srRNA using polymerase chain reaction (PCR), revealed that all *S.aureus* isolates were positive (100%) and the results of resistant gene femA revealed that among 2 isolated belong to pathogenic *S.aureus* one isolate contains this gene (50%), whilst 2 isolates belong to S.aureus from the environment do not contain this gene (100%) . The PCR result for the nuc gene indicated that among each 2 isolated belong to pathogenic (clinical) S.aureus isolate one (1) isolate contain this gene (50%), whilst 2 isolates belong to *S.aureus* from the environment do not contain this gene (100%).
Conclusion: Resistant gene femA get found in pathogenic S.aureus one isolate contains this gene , whilst isolates belong to S.aureus from the environment do not contain this gene , the nuc gene get found in pathogenic (clinical) S.aureus isolate one (1) isolate contain this gene , whilst isolates belong to S.aureus from the environment do not contain this gene .
Key words: *Staphylococcus aureus* , virulence genes, water, soil, hospital, PCR.
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Introduction

Staphylococcus aureus are isolate nurses and serious task because of the ability to cause various types of injuries at different locations of the body, and that pathogen has

nothing to do with their ability to produce many factors of Virulence to produce toxins and enzymes extracellular enzymes which help extracellular bacteria and overburden diplo and spread within host tissues, plus high resistance and multiple anti-B-lactam and aminoglycosaid which in turn makes them major nurses Of Nosocomial infection [1].

Staphylococcal is of the most prevalent bacteria in nature, as there is in the air , soil ,on human skin , mucous membranes , respiratory tract , pathological form exists in human nasopharyngitis, cause serious injuries, these bacteria from skin infections and may execute to blood and all members of the body, causing blood poisoning and infections of heart valves and other diseases [2]. The pathogenesis of these bacteria and its ability to invade host tissue and spread it back to owning a lot of virulence factors as capsule which help resist bacteria for the process of phagocytosis, besides owning cell wall that runs on the host's immune system resistance [3]. Some breeds have mucosal layer helps process is incomplete, and bacterial colonization with host cells, in addition to its ability to produce many toxins and extracellular enzymes that help bacteria in causing injury. Like the coagulating enzyme for blood plasma that has the ability to inhibit the process of phagocytosis, as well as their ability to produce other enzymes were spread (Spreading Factors) as the enzyme stophylokinase,protase, lipase, which contribute to the invasion of bacteria to tissues, and the spread of infection and run on toxins a blood analyst production alpha and beta type gama and delta, along with intestinal toxins production leads to food poisoning [4].

16srRNA gene

Uses a 16srRNA gene and commodity prices and the primitive Prokaryotic species diagnosis routine [5], and uses the gene diagnosis of bacteria and rated and determine kinship relations between races and species, as well as the relationships between very old people [6]. 16srRNA uses in diagnosis and classification of bacteria for several reasons:-

1- It is found in most types of bacteri.
2- The gene function is stable and do not effect by the time
3- Total length of the gene is about16srRNA (1500 bp) and its is long enough for the purpose of carrying genetic information [7] .

Fem A gene

Gene femA is the multiple genes responsible for resistant bacteria for Methicillin and generally features found in all sorts of S.aureus resistance MRSA. FemA 48KD protein gene produces a protein involved in cell wall metabolism and is found in large quantities in actively developing communities [9; 8]. FemA is also one of the necessary genes responsible for rapid cycle decomposition self autolysis [10]. Bio-chemical analysis also suggested that production may also involve femA gene synthesis cell wall [11].

Nus gene

Gene nus (thermonnusclease) uses to select and discover S.aureus as known as gene

S.aureus species specialist [12]. encrypt the gene for the thermonuclease and is used widely as a specialist to identify S.aureus by PCR [13 14;]. nuc is extracellular nuclease [15] this gene always used as a molecular target to identify S.aureus [16]. gene nuc selected for detection of enterotoxigenic produced S.aureus So highly correlated nuc gene showed the production of internal toxins[17].

Patients and Methods

I attended a series of solutions decimals by adding 1 g of each sample of soil samples to 9 ml of normal saline was and 1 ml of water samples and add to 9 ml of normal saline . Cultivated forms directly after a solutions by adding 0.5 ml of twenty alleviation II to the surface of Nutrient agar medium and incubated in temperature of 37 c˚ for 24 hour . then it was testing and diagnostic morphology and biochemical of bacterial isolates [18].

Isolates were obtained Staphylococcus aureus bacteria back of Microbiology Laboratory in hospital diagnostic was conducted education through the work of some phenotypic, biochemical tests, so add some special tests were performed staph like on Mannitol salt agar and the Coagulase enzyme plasma screening test.

Bacterial isolates were subjected to microscopic examination gram stain taking a small tinge of colony and do gram dye to see bacteria interact with the dye and the form and manner of cells assembled [19]. biochemical tests were performed to diagnose bacterial isolates the level type as indicated in [20].

Bacterial DNA extraction

Bacterial DNA was isolated using several extraction (Bacteria DNA Mini kit) fitted from Bionner company and follow the following steps:

1- Transfer 1 ml of bacterial isolates the development 24 hours to eppendorf 1.5 ml.
2- Eppendorf tubes discarded central pipe for min 14000 rpm, floating neglected.
3- Added 200 microliter of GT buffer to eppendorf tubes and mix it thoroughly .
4- Added 20 microliter of the enzyme proteinase K record in accordance with paragraph (4-1-2-3) into the pipe and then merged mediated vortex mixer well, then placed inside a water bath for ten minutes and the temperature 60 º c and heart pipe three times every three minutes during the cuddling.
5- Added 200 microliter of GB buffer into the pipe and then merged with the mediation well vortex mixer for 10 seconds and then placed inside a water bath Water bath for ten minutes and the temperature to 70 º c and flipped the pipe three times every three minutes during the cuddling.
6- Added 200 microlitr of absolute ethanol Absolute cold to piping and then mix gently through the heart of the pipes to precipitate DNA.
7- The column is placed in the collection tube Column GD Collection tube 2 ml and transfer all the batter.

8- Central pipe renounced for two minutes fast 14000 RPM then contents to new collection tube.
9- Added 400 microlitr of W1 buffer to GD column.
10- Mixture pipes quickly jettisons 14000 rpm for 30 seconds then put GD column in a tube.
11- Added 600 microlitr of Wash buffer to GD column.
12- Run the central profiles for a minute 14000 RPM.
13- Give central profiles for three minutes fast 14000 rpm for a minute to dry the column.
14- Move the column GD to clean abendrov tube 1.5 ml
15- Add 50 micoliter of Elution buffer heater 70 degree º c to pipes and pipe left for five minutes, then fired centrally for half minute 14000 rpm speed so that DNA is obtained.

Gel Electrophoresis

Run the migration of DNA extracted according to (21) and as follows:

attended gel agaros 1% concentration of melt (1 g) of agaros (100 ml) of saline solution 10 x TBE after diluted 10 times to get 1x TBE, hot the agaros to a boil and leave to cool down to Temperature (45 º c), then added to a final concentration of the ethidium bromide dye 0.5 micrograms per/ml using the storage solution to record this dye then mix well the agaros to base plate set tray briefed his dissatisfaction about duct tape and well, then comb proved to configure drill Wells designed to download samples and then pour agaros quietly and continuously to avoid air bubbles, then leave the gel solidifies at room temperature, and calmly comb for Duct tape. Then move the gel with the template to outdoor posting containing a suitable size covers jellies of TBE 1x then got carry Samples for posting (5 microlater) of DNA template and then mixing it with (3 microlater) 6x download buffer using micropipette . After the migration process is made power supply voltage of 100 v/cm for (45 minutes) until the arrival of the samples before the end of the gel, after completing the migration process transferring the action to exposed to the source of the radiation exposure in violet. UV-Transilluminator when wavelength 320 nanometers.

Polymerase Chain Reactio

Gene was detected using PCR technique and follow the following steps:

1- Mix Master President attended the interaction mixture and add the following ingredients in a sterile tube as in table (1) mix the ingredients with a glass micro pipette minute by dragging it several times and then put in your profiles for 10 seconds.
2- Spread the mixture on the sterile tubes 0.5 ml size 8 and mentor as samples to be examined and 15 µL of the mixture for each tube.
3- Add (5 µL) from a DNA template of each sample into the tube of his custom, the distributed Master Mix on the pipe before transfer of DNA to avoid contamination.

4- Use centrifuge for (15 seconds) to collect drips Seagrasses Central expulsion pipe wall and relegated to down for the tube keeping the final size of a mixture of interaction 20 µL.

Optimal conditions of femA, 16srRNA, nuc gene amplification

Replication steps were performed to investigate the level of S.aureus bacteria and detection femA, 16srRNA, nuc gene according [22] PCR device is programmed, as shown in table(2).

Results

Tested four samples of S.aureus bacteria that carry sequences (1-5-23-36), having chosen these samples for genetic diagnosis by 16srRNA gene and detection of virulence genes and genes exist femA, nuc, using a technique (PCR) and rely on Primers specialized femA genes, nuc and fitted by a company (Korea/Bioneer) according to the sequence designed by (22 , 23), table (3). have been programming device (PCR) as shown in the table (2) and the use of DNA template extracted by (Bacteria DNA Mini kit) is equipped by Bioneer Corporation, and has been detected Bacterial DNA extraction products using electric agaros gel, and use the optical density measurement device Spectrophotometer with purity of samples ranging from (2-1.8). nomads your output sequence polymerase 16srRNA gene on agaros gel focus (1%) (1) voltage (Volt), noting the emergence of one package of all pits after exposing to UV gels as the picture (1). This demonstrates the link initiator with complementary sequence to tape the template when comparing packages with volumetric Ladder DNA evidence-doubled is equipped by a company (Korea/Bioneer), noted that the package sizes similar to expected size is (1400 pb), current study [4] S.aureus bacteria samples were have 16srRNA, as well as posting results showed a Electrical resistance genes femA, nuc said that all environmental S.aureus isolates were non-container on this genes by (100%) . For pathogene S.aureus isolates isolation was one of total 2 isolation used in study on gene femA container (50%) and output packet size (509bp) and is identical to the expected product size (509bp) as for the nuc gene was isolated was one of total 2 isolation used in study on container nuc gene percentage (50%) and the resulting packet size (397bp).

Table (1): The main interaction Master mix components.

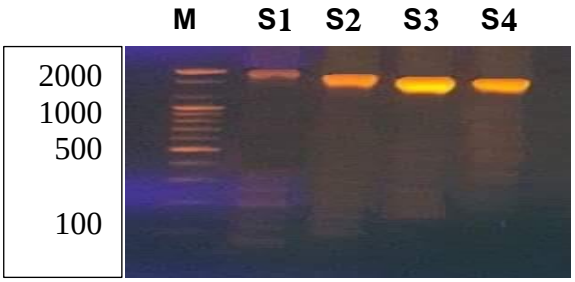
Final Concentration	Volume For 1 Tube	Materials Concentration Manufacturer
……..	6 µL	D.W
1x	5 µL	Accupower ® PCR Premix
10 pmol	2 µL	Primer – F (10 pmol)
10 pmol	2 µL	Primer – R (10 pmol)
µL15		Total reaction Volume

Table (2): Program of fmeA, 16srRNA, *nuc* gene amplification, P.C.R technology.

Type of primers	PCR Conditions Temperature / Minute								
	Denaturation				Annealing		DNA-extension		Expected size of products (bp)
	One cycle		40 cycles						
	°C	Min	°C	Min	°C	Min	°C	Min	
16srRNA	94	5min	94	1min	42	1min	72	5min	1400
fmeA	94	3min	94	1min	60	1.30 min	72	2 min	509
nuc	94	3min	94	1min	60	1.30 min	72	2 min	397

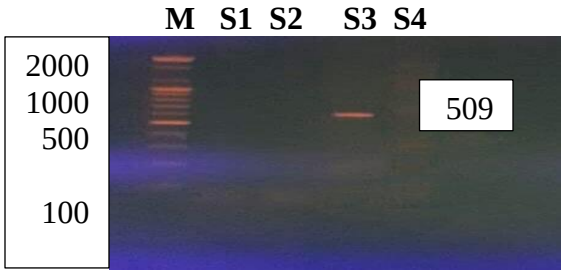
Table (3): Shows the variety and volume sequences expected output for each prime.

Product size	bp Base pair	Primer sequence 5⁻→3⁻	primer
1400	20	5-AGAGTTTGATCCTGGCTCAG-3	16-srDNA-F
	15	5-CAAGGCATCCACCGT-3	16-srDNA-R
509	20	5-AGACAAATAGGAGTAATGAT-3	FemA-F
	20	5-AAATCTAACACTGAGTGATA-3	FemA-R
397	21	5-CTGGCATATGTATGGCAATTG-3	Nuc-F
	20	5-AATGCACTTGCTTCAGGACC-3	Nuc-R



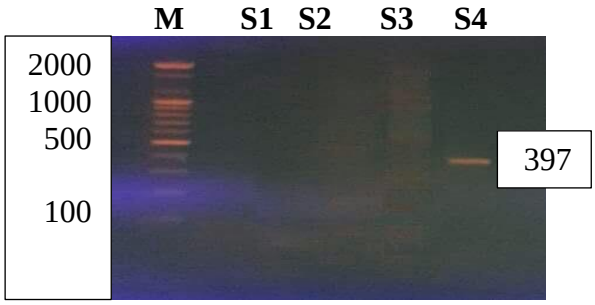
Picture (1) : The Electrophoresis of 16srRNA gene on agarose gel using PCR for *S.aureus* , 1% , 100 volts for one hour.

(S1) = Sample (1) of pathogenic S.aureus , (S2)= Sample (5) of pathogenic S.aureus , (S3)= Sample (23) of environmental S.aureus (S4)= Sample (36) of environmental S.aureus , (M)= Marker 100-2000pb .



Picture (2) : The Electrophoresis of *fe m*A gene on agarose gel using PCR for *S.aureus* , 1% , 100 volts for one hour**.**

(S1) = Sample (23) of environmental S.aureus , (S2)= Sample (36) of environmental S.aureus , (S3)= Sample (1) of pathogenic S.aureus (S4)= Sample (5) of pathogenic S.aureus , (M)= Marker 100-2000pb.



Picture (3) : The Electrophoresis of *nuc* gene on agarose gel using PCR for *S.aureus* , 1% , 100 volts for one hour.

(S1) = Sample (23) of environmental S.aureus , (S2)= Sample (36) of environmental S.aureus , (S3)= Sample (1) of pathogenic S.aureus (S4)= Sample (5) of pathogenic S.aureus , (M)= Marker 100-2000pb.

Discussion

The result of 16srRNA compared with the results of the [23], gene 16srRNA, that Widely used to diagnose bacterial species and is used in classification and learn the relationships between species and this gene is not homogeneous and identical in all living where there are a range of ambient Lunar occurring during the process of evolution [24], and when we compare the result of femA , nuc genes with presenting her findings [22], as well as posting results showed electric resistance genes nuc, femA that all environmental S.aureus bacteria isolates by (100%) the results showed that all isolates were non-container on this gene and this result does not correspond with presenting her findings [22] to him in Iraq, which was the source of his retirements of blood and urinary tract infections with all the container isolates gene femA (100%) And gene nuc (20%). For pathogene S.aureus isolates isolation was one of total 2 isolation used in study on gene femA container (50%)

and output packet size (509bp) and is identical to the expected product size (509bp) this close with the findings (22) if all isolates The container has gene femA (100%). as for the nuc gene was isolated was one of total 2 isolation used in study on container nuc gene percentage (50%) and the resulting packet size (397bp) and is identical to the expected product size (397bp) this close with the findings (22) if 2 isolated from the total 10 isolates percentage (20%) on the container this gene . And different results due to differences in the source and type of samples.

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