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Molecular identification of methicillin-resistance *Staphylococcus aureus* isolated from milk of breast infection woman in Diwanyia province.

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Abstract

Methicillin-resistance *Staphylococcus aureus* (MRSA) is the greatest important source of community-acquired infection in humans. The present study was carried out to use (PCR) assay as more specific molecular methods for recovering of methicillin resistant *S.aureus* (MRSA) isolates that isolated from milk of breast infection woman which collected from Diwanyia hospital . PCR assay was based on used specific primers that amplified of *mecA* gene in *S.aureus* isolates. The primers were designed in this study by using NCBI-Genbank data base (KM505042.1) and primer 3 plus primers design. The results of primary identification bacterial isolation were show 16 positive *S.aureus* isolates out of 50 milk samples. From those 5(31.25)were identification methicillin antibiotic resistant *S. aureus* (MRSA) using PCR assay The conclusion was to establish a fast and specific PCR tool for the detection of methicillin resistant *S. aureus* (MRSA)in human milk that may be major risk factor human health.

Key word: Staphylococcus aureus, MRSA, PCR, Milk

Introduction

Staphylococcus aureus is a main important bacterial pathogen in both hospitals acquired infection and the community acquired infection [1]. It is a member of family *Micrococcacea*, it be Gram positive cocci, and itis occurs in either singly coccus or form pairs, or which show as clumps of cluster “grape-like” [2]. *S. aureus* is commonly colonized on external skin surfaces particularly the nasal passages in Human. This bacterium is opportunist pathogen, and can be cause more severe after burns woundbacteria infections, the bacterium which invaded the tissue and production of toxins e.g. toxic shock syndrome [3]. Methicillin resistance *Staphylococcus aureus* is clinical infection isolates which arise from the production of β-lactamase enzyme that hydrolysis of methicillin [4]. The methicillin is member class of β-lactamase antibiotic, that inhibiting cell wall formation which comprised in the peptidoglycan synthesis mechanism and composed mesh like polymer which protect and surrounds the bacterial cell [5]. Methicillin resistant *S. aureus* (MRSA) are actuality recorded with high frequency in the public community and they have been named community-acquired (MRSA), mostly associated with soft tissue and skin infection [6]. The presence of Methicillin resistance *S. aureus* (MRSA) in specimens of human breast milk can be description for secondary contamination from surrounded skin, breasts and the contamination nasal cavity of baby donor [7]. Many studies reported that the occurrenc of (MRSA) from human milk, and some of these strain capable to cause disease of mastitis by production exotoxins [8]. Other reports on (MRSA) especially in postpartum mastitis mostly among young, while the other healthy milking women lack risk of factor for (MRSA) that arisen in the previous a small number of years [9][10]. Molecular techniques have the potential of offering highly sensitive and rapid in detection and identification of mecA gene in *S. aureus* with Methicillin-Resistans. Therefor our study was aimed to molecular detection of (MRSA) which isolated from milk specimens of breast infection woman in Diwanyia province.

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Materials and Methods

Milk specimens **collection:** 50 specimens of milk of breast infected milk woman were collected in Al-Diwanyia hospital. The milk specimens were collected in 25ml sterile containers after clean and washing the breast using disinfectant solution, then the milk specimens transported into laboratory and stored in a refrigerator until use for bacterial stady.

Bacterial Isolation: *Staphylococcus aureus* was isolated from milk specimens by inoculation on Brain Heart-Infusion Broth (BHI) at 37°C for overnight for primary enrichment culture and the growth of bacteria were inoculated on mannitol salt agar (MSA) at 37°C overnight for selective isolation of pure culture *S. aureus* isolates. After that, the positive growing *S. aureus* isolates were confirmative identification using (VITEK 2 Compact, Biomerieux).

Bacterial DNA extraction and PCR Method:

PCR technique was performed for detection Methicillin-Resistant *Staphylococcus aureus* basd mecA gene in *S. aureus* isolated from milk specimens by following steps:-

1-DNA extaraction: The *Staphylococcus aureus* isolates were subjected to nucleic acid extraction using commercial total DNA extraction kit (Presto Mini-DNA Bacteria Kit. Geneaid Biotech Ltd.USA) The extraction method was don depend on the manufacturing instructions using gram positive bacteria DNA Protocol extraction method using (20 mg/ml) lysozyme buffer.

2-Nanodrop: The extracted DNA was estimatd by nanodrop device at 260/280nm, and then kept at deep freezer until used in PCR technique.

3-Primers : The primer of PCR that is useing in this study for deteced Methicillin-Resistant *S. aureus* based on mecA gene were designed in NCBI Gene sequnce data base (Genbank code: KM505042.1) and primer 3 plus design. These primers were provide from «Bioneer company, Korea» as following toble(1):-

Table (1) :- PCR primrs mecA gene:

Primer	Sequence (5'-3')		Amplicon
mecA gene primer	F	TCTTGGGGTGGTTACAACGT	503bp
	R	TTGAGTTCTGCAGTACCGGA	

4- master mix preparetion of PCR: the mix is prepar using «Accu-Power®PCR-Pre Mix-Kit» master mix reagent and done depend on company instructions as following table (2) Tabla (2) company instructions of PCR master mix:-

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Master mix	Volume
DNA template (10ng/µL)	5µL
Forward primer (10pmol)	1 µL
Reverse primer (10pmol)	1 µL
PCR water	12 µL
Total volume	20

next, the mixof PCR that revealed within table on top of placed in AccuPower (PCR –Pre) Mix that contain all other PCR workings which needed to reaction such as « Taq DNA polymerase, dNTPs, 10 PCR buffer ». at that time, all the PCR tubes transferr into vortex centrifuge« for 3 minut». Then transferred into thermocycler «My Gene, Bioneer. Kore»

5- PCR thermocycler conditions:

Table (3) PCR thermocycler conditions

PCR step	Temp.	Time	repeat
Initial Denaturation	95C	5min	1
Denaturation	95C	30sec.	30 cycle
Annealing	57.2	30sec	
Extension	72C	1 min	
Final extension	72C	5min	1
Hold	4C	Forever	-

6- PCR product analysises: The PCR products (503bp) were examined by electrophores in a 1% agaros gel using 1X TBE buffer, stained with ethidiume bromide , plus investigation under U V transilluminator.

Results :

the specimens were firstly inoculated on on Brain Heart-Infusion Broth (BHI) and a total of 16**(32%)**the growth of bacteria were inoculated on mannitol salt agar (MSA) The colonies appeared on the (MSA) pale yellow and gold color ,After that the positive growing *S. aureus* isolates were confirmative identification using (VITEK 2 Compact, Biomerieux).

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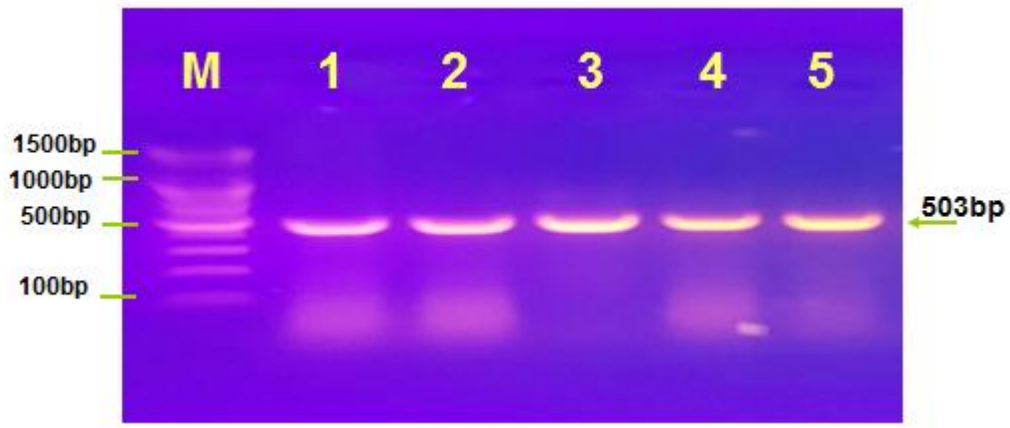
The PCR was appeared as highly sensitive and more specific assay in direct detection of Methicillin- resistant *S. aureus* (MRSA) from *S. aureus* isolated milk infacted breast woman as following table:

Test	No. of Tested specimens	Positive samples	Percent
Bacterial isolation	**50**	**(16)**	**(32%)**
PCR	**16**	**5**	**31.25%**

The PCR detection of mecA gene in5(**31.25%**) *S. aureus* isolates samples was shown good PCR product bands on examination by gel electrophoresis at 503bp product size. Figure (1).



Figur (1): Electrophorsis image of PCR products of the positiv results of mecA gen in Methcillin -rasistant *S. aureus* (MRSA) from breast milk at 1% agaros gel. Where, first well lane (M) DNA ladder marker (1500-100bp) , other well lane (1-5) postive isolates at 503bp PCR product.

Discussion :

In this investigation, a total number of 50 specimens were subjected to bacteriological examination for detection and isolation of *S. aureus* isolates. The Developing colonies appeared on the central Mannitol Salt Agar in yellow, pale yellow, golden, reaching diameters (1-2) milli meters, in addition to the center color of red was changed to yellow color due to the fermentation of mannitol and acid production These results were consistent with the mention[11],[12],[13]. The Incidence of *S. aureus* among the examined specimens was readed 16(8%) isolates and can be attributed of the high rates of *S. aureus* clinical samples to the fact that man is the center of a development rich in bacteria that you need and supplied to the

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temperature and humidity had the appropriate growth and reproduction and that an exceptionally versatile organism were adopt many ecological nichesand in spite of the presence of defensive means, immune to the human body but the Staphylococcus aureus You can find numerous citizen to breed long-term colonization[11].

The polymerase chain reaction (PCR) technique is found very specific and sensitive assay, and less time consumption, when using for direct detection of methicillin-resistant *Staphylococcus aureus* from breast milk, in contrast to other conventional diagnostic techniques.

Our results showed that PCR is very important tool for the identification of (MRSA) from milk by using universal primers for of mecA gene in methicillin-resistant *Staphylococcus aureus* , These results were in agreement with [14]who referred that 21 cases with methicillin resistance, 17 (81%) occurred in 2005and the increase is due tocommunity-acquired methicillin-resistant *S.aureus* ,in[15] MRSA has emerged in patients without established risk factors,that MRSA infections by (15%) were community-associated and (85%) were health care-associated.

Results indicated shown in table 1Samples of milk of breast infection woman This is consistent with a study[16],where he reached, 45.1% of S. aureus isolates were mecA- positive in the PCR assay. in another study, the researcher found from being isolated from human patients with mastitis with more reports indicate that between 30-50% of bacteria from human human mastitis is S. aureus [17]while (MRSA) is a relatively common result in human clinical mastitis rights this represents threat to for the patient. MRSA importance came through the resistance of many antibiotics, such as resistance to all types of anti-B-lactam antibiotics and may cause a problem for patients and their treatment and thus failed to control the infection occurs [18].

This case indicates the growing somewhat fear in bringing different injuries in the body, and difficult to treat, control and dissemination of the pathogen in the population and the environment. So as to anticipate the likely effects on further necessary public research to understand the dynamics of the diseas from S. aureus, especially in the city of Diwaniyah hospitals..

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