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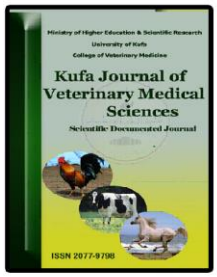

Direct detection of "methicillin resistant Staphylococcus aureus" from buffalo raw milk in Al-Qadissiya province using Polymerase Chain Reaction assay

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Abstract

The shedding of "methicillin resistant *Staphylococcus aureus*" in raw buffalo milk causes a potential risk if consumed without maintaining sufficient hygienic criteria due to numerous clinical implications to human infection. The present study was used the Polymerase chain reaction technique as highly specific molecular procedures for direct detection of "methicillin resistant *Staphylococcus aureus*" (MRSA) of buffalo raw milk samples that compiled from animals owners of different areas in the province of Diwaniyah during the period from July 2016 to December 2016. PCR technique was dependent on used specific primers that amplification of mecA gene in *Staphylococcus aureus*. This primer was designed in this study by using NCBI-Genk data base (KM505043.1) and primer 3 plus for primers design. The PCR results were shown that buffalo infected with (MRSA) at 8 positive samples at percent (16%) out of 50 milk samples.

The purpose of this research was to establish a rapid and specific PCR Technique for the diagnosis of "methicillin resistant Staphylococcus aureus" in buffalo raw milk that will be used as alternative to the currently available convention detection methods and makes it possible to identify the foods at risk for MRSA contamination to public health.

Key wards : *Staphylococcus aureus* , PCR , MRSA, buffalo

التشخيص المباشر لبكتريا "المكورات العنقودية الذهبية المقاومة للمثيسيلين" من عينات حليب الجاموس الخام في محافظة القادسية باستخدام تقنية فحص تفاعل سلسلة البلمرة

الخلاصة

ان طرح جرثومة المكورات العنقودية الذهبية المقاومة للميثيسيلين في حليب الجاموس الخام يسبب خطرا محتملا على صحة الانسان إذا استهلك دون الحفاظ على معايير صحية كافية. وقد استخدمت الدراسة تقنية تفاعل البلمرة المتسلسل للكشف المباشر عن المكورات العنقودية الذهبية المقاومة للميثيسيلين من عينات حليب الجاموس الخام والتي تم جمعها من المربين من مختلف المناطق في محافظة القادسية خلال الفترة من يوليو

2016 إلى ديسمبر 2016 حيث تم جمع 50 عينة من حليب الجاموس الخام ,و باستخدام برايمرات خاصة للجين الذي يشفر بروتين الصدمة الكهربائية , صممت البرايمرات المستخدمة بالدراسة اعتمادا على موقع بنك الجينات العالمي
لتصميم البرايمرات . primer 3 plus و NCBI-Genk data base (KM505043.1)
اظهرت النتائج ان 8 عينات موجبة بنسبة 16% من حليب الجاموس مصابة بهذه الجرثومة.
كان الغرض من هذا البحث هو ان تقنية PCR تقنية سريعة ومحددة لتشخيص المكورات العنقودية الذهبية المقاومة للمثيسيلين في حليب الجاموس الخام التي سيتم استخدامها كبديل للطرق التقليدية للكشف عن بكتريا (MRSA) ويجعل من الممكن تحديد الأطعمة الملوثة بهذه البكتريا وبالتالي تحديد خطر التلوث على الصحة العامة.

الكلمات المفتاحية: المكورات العنقودية الذهبية, الجاموس, MRSA , PCR.

Introduction

The worry that constitutes a threat to public health is the source of the emergence of microbiology antibiotic resistance in environment including farm animals (1).

The significant opportunistic pathogen in both of humans and in livestock is *Staphylococcus aureus* and causes zoonotic disease and it be revealed primary reason of a chronic, subclinical mastitis in dairy cattle worldwide (2).

Staphylococcus aureus is a common strain in research studies because it is the most pathogenesis of the 32 species and eight 'sub-species' belong to the genus Staphylococcus (3),these increasing importance of *Staphylococcus aureus* due to the elevation in it's antibiotics resistance (4).

"Methicillin-resistant *Staphylococcus aureus"* (MRSA) has appeared as a main reason of the "community-associated (C A)" infections and "health care-associated (H A)" (5).

Healthy person possible to be a holder of a bacterium (MRSA) without clinical signs for intervals ranging between a few weeks to several years, "S.aureus" most usually inhabits under the frontal nares (the nostrils). The unlocked injuries and urinary tract are also latent situates for infection in human and animals(6), (7).

The basic element in the human diet is milk, but milk at the same time is a suitable environment for microbial growth, especially pathogenic bacteria include ("Salmonella", "*Listeria monocytogenes*", *"Staphylococcus aureus*", "Campylobacter", "Yersinia") , the buffalo milk is rich in carbohydrates, fats, therefor these components considered nutritious elements for growth of the bacteria staphylococcus aureus (8),(9).

This study presented aims to isolate and diagnosis "methicillin resistant Staphylococcus aureus" (MRSA) from raw buffalo milk samples of different regions in Al-Qadissiya province by using polymers chain reaction (PCR).

Materials and Methods

Samples collection: Collected 50 buffalo raw milk samples were compiled from animals owners of different areas in the province of Diwaniyah within the period from July 2016 to December 2016. The samples were collected in 10ml sterile tube and transported into place examined (laboratory) and stockpiling by

freezing until use for "genomic D N A extraction".

Bacterial "DNA extraction" and PCR Method:

P C R technique was performed for detection "Methicillin-Resistant *Staphylococcus aureus*" based mec A gene in *"Staphylococcus aureus*" isolated from milk samples by following steps:-

1-D N A extraction: The milk samples were subjected to bacterial nucleic acid extraction by using commercial DNA extraction kit (Presto Mini-D NA Bacteria K it. Geneaid Biotech. L td. U S A) and according to method described by Sreevatsan **.** The extraction method was don depend on the manufacturing instructions by using gram positive bacteria D N A Protocol extraction method by using "20 mg / ml" lysozyme buffer.

2-Nano drop: The extracted DNA was estimated by "nanodrop device" at 260 /280 n m, and then kept at deep freezer until used in P C R method.

3-Primers: The PCR primers that used in this study for detection "Methicillin-Resistant Staphylococcus aureus" based on mec A gene were designed in this study using N C B I Gene sequence data base (Gen bank code: KM505042.1) and primer 3 plus design. These primers was provided from Bioneer company, Korea as following table (1):-

Table (1) :- PCR primers mec A gene:

Primer	Sequence (5'-3')		Amplicon
mecA gene	F	TGGCCAATACAGGAACAGCA	426bp
	R	CGTCAACGATTGTGACACGA	

4- The "PCR master mix preparation": the mix was attended by using (Accu-Power® P CR-Pre Mix-K i t) master mix reagent and done depend on company instructions as following table(2):-

Table (2) company instructions of PCR master mix

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

After that, the P C R mix that revealed in table above placed in Accu Power P C R – Pre Mix that contain all other PCR components which needed to reaction such as (Taq "DNA polymerase", "dNTPs", 10 PCR buffer). Then, all the P C R tubes transferred

into "vortex centrifuge" for 3 minutes. Then transferred into thermo cycler (My Gene, Bioneer. Korea).

5- PCR thermo cycler conditions:-

Table (3) PCR thermo cycler 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- P C R product analysis: The P C R products (503 b p) were examined by electrophoresis in a 1% "agarose gel" using "1X TBE buffer", stained with "ethidium bromide", and conceive under "U V transilluminator".

Results and Discussion

The Polymerase chain reaction P C R was appeared as sensitive and specific assay in direct diagnosis of MRSA from buffalo raw milk as following table(4):

Sample	No. of Tested samples	Positive samples	Percent
Raw milk	50	(8)	(16%)

The P C R amplification of "mecA gene" in positive samples was Illustrates clear PCR product bands on "Agarose gel electro phoresis" at 426 b p product size products. Image (1)

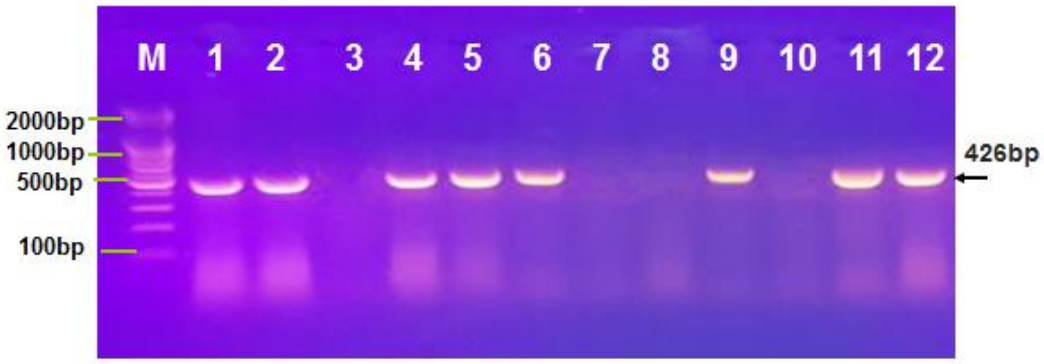

Image (1): display "Agarose gel electro phoresis" of PCR reaction, the positive results of "mecA gene" of (MRSA) from buffalo raw milk. In which, Lane (M) DNA marker (2000-100bp), Lane (1,2,4,5,6,9,11,&12) positive samples at "426 bp" PCR product.

The bacteria that responsible for many of human diseases which difficult treatment is "Methicillin-resistant *Staphylococcus aureus*",(MRSA) is any strain of "*Staphylococcus aureus*" that has multi resistance to beta - lactam anti biotics, it includes the penicillins (" Methicillin", " Dicloxacillin", " Nafcillin", " Oxacillin", etc…) (10).

In Iraq,(11) recorded 22 isolates of *Staphylococcus aureus* from 215 buffalo milk samples, after that examined eight isolates by using sensitivity of antibiotics , showed results 100% resistant to oxacillin and ampicillin, this result higher than findings of the current study, also study of (12) in Iraq, recorded high percent (82.47 %) of (MRSA) from 200 *"Staphylococcus. aureus"* isolates which insulated from milk and white cheese samples of local shops in Baghdad province and (13) showed in his study there are 10 isolates of MRSA in the rate (33.4 %) of 30 milk and white crude soft cheese samples that collected randomly from different markets in Baghdad Province as 4 isolates from raw milk in the rate of (13.4%) and 6 isolates from white raw soft cheeses in the rate of (20 %).

The study of (14) in India also reported high percent (24%) of (MRSA) than current study, this high percent duo to varying unhygienic conditions such as improper cleaning of utensils, dirty udders, animals with sub-clinical mastitis, milk handling techniques and improper/lack of refrigeration and storage that are known to increase the proportion of *Staphylococcal* spp. in raw milk and milk products.

As well as study of (15) in northern Morocco who found that the percentage of *Staphylococcus aureus* resistant to oxacillin was 85% from raw milk and milk products.

But (16) recorded lower percentage of (MRSA) in raw milk from shops in Multan city in Pakistan (10.39 %) ,also study of (17) recorded lower percentage than the current study which was (4%) .

Study of (18) reported 14 positive sample of MRSA from 107 samples of *"Staphylococcus. aureus"* isolates in the rate (13 %) from cattle , which is Compatible with findings of present study.

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