MOLECULAR CHARACTERIZATION OF PSEUDOMONAS AERUGINOSA ISOLATED FROM MILK

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ABSTRACT

A total of 200 samples of raw and pasteurized milk (100 of each) were collected from supermarkets in Qaluobia Governorate and examined for prevalence and characterization of *P. aeruginosa* .The incidence of *P.aeruginosa* isolated from raw and pasteurized milk was 40 %and 16%, respectively. Serogrouping of *P.aeruginosa* isolated from examined samples revealed that serogroupes K, H and, A isolated from raw milk at frequency percentage of 40, 35, and 25, respectively, while from pasteurized milk at frequency percentage of 25, 50 and 25, respectively. For the detection of the enzyme Hameolysine, Phospholipase, Caseinase and Gelatinase revaled that for serogroupe K it were 60,50,100 and 90 % respectively while for serogroupe H it were 54.5,45.5,100 and 81.8 % respectively and it were 42.8,28.5,100and85.7% for serogroupe A, respectively. Concerining the pathogenicity test of *P.aeruginosa* isolated from milk samples it was in percent 100, 100 and 57.14 for serogroupes K, H and A, respectively. *P.aeruginosa* varied in its resistance to different antibacterial agents, ciprofloxacin was the most effective drug against *P.aeruginosa* (60.7%), followed by enrofloxacin, norfloxacin and lomefloxacin (53.6%). *P.aeruginosa* was low in susceptibility to tobromycin (25.0%), gentamycin (21.4%) ceftriaxone (17.9%) streptomycin (10.7%) erythromycin (3.6%) tetracycline (3.6%). The organism was completely resistant to trimethoprim, naldixic acid and doxycen. For identification of *P. aeruginosa* strains at the DNA level, Polymerase chain reaction (PCR) is used based on specific primer for 16S rRNA, PCR has found to be rapid and more sensitive and specific in identification of *P. aeruginosa,* and the bands appeared at 956 bp.

Key words: *Molecular characterization, P. aeruginosa, Milk.*

INTRODUCTION

Milk is highly prone to contamination and can serve as an efficient vehicle for human transmission of foodborne pathogens, especially gram-negative bacteria, as these are widely distributed in the environment (Garedew *et al.,* 2012). Such balanced diet becomes contaminated with several types of microorganisms which originate from the soil, water or skin and the hair of the animals or utensils or from the milk handlers (Lendenbach and Marshal, 2009).

Genus Pseudomonas is the most heterogonous and ecologically significant group of known bacteria, and includes Gram-negative motile aerobic rods that are wide-spread throughout nature and characterized by elevated metabolic versatility, thanks to presence of a complex enzymatic system. The nutritional requirements of Pseudomonas spp. are very simple, and the genus is found in natural habitats like soil, fresh water, and marine environments. (Aysel *et al.,* 2012).

Pseudomonas spp. plays an important role in milk spoilage. During the storage of raw milk they produce many thermo-tolerant lipolytic and proteolytic enzymes that reduce both the quality and shelf life of processed milk (Wiedmann *et al.,* 2000).

It is an opportunistic pathogen that can affect human, animal and birds. Amany *et al.,* 2006). Temperature plays a major role in the diversification of microorganisms and spoilage of milk during transport, processing and storage. Psychrotrophic bacteria have been recognized as a recurring problem in the refrigerated storage and distribution of fluid milk, and perishable dairy products for several decades (Singh *et al.,* 2012). So, the psychrotrophs have received increased attention by investigators during recent years, because modern developments in the handling and transportation of milk have resulted in milk being held for longer period at refrigeration temperature before processing, manufacturing or consumption (Olfa *et al.,* 2013).

Psychotropic pseudomonas is recognized as major spoilage microorganisms based on their extracellular thermo-tolerant lipolytic and proteolytic enzymes. The enzymes are generally good indicator of the keeping quality of protein and lipid-rich foods (Cousin *et al.,* 2001).

During the storage of raw milk this enzymes reduce both the quality and shelf life of processed milk (Dogan and Boor, 2003). Although pasteurization of milk has been practiced as the most effective method of reducing the risk of contamination and spreading of disease, many of these enzymes can survive pasteurization (72°C for 15 s) and even ultra-high-temperature treatments (138°C for 2 s or 149°C for 10 s) and can thus reduce the sensory quality and shelf life of processed fluid milk products (Arslan *et al.,* 2001). Second, post pasteurization contamination contributes most of the microorganisms, primarily Pseudomonas spp., that cause spoilage of conventionally pasteurized milk during refrigerated storage (Laura and Mauro 2007).

Although microbial spoilage may merely lead to foodstuffs being rendered unpalatable, it can also result in serious and even fatal illness (Hogg, 2005) *Pseudomonas aeruginosa* is pathogenic for humans and animals, often as a secondary infection It is now recognized as a common source of many community acquired and nosocomial infections, and affects primarily immunocompromised people and those suffering from cystic fibrosis. The most common infections involve the cornea giving rise to a blue green pus, skin, urinary tract, brain and respiratory tract (Corona *et al.,* 2001).

Infections caused by *P. aeruginosa* are particularly problematic because many aerobic pseudomonades are resistant to several antibacterial agents (Moore *et al.,* 2006). This subject is of particular medical importance because members of this group, such as *P. aeruginosa,* are serious opportunistic human pathogens (Yahr and Parsek, 2006).

The effective antimicrobial agents for the treatment of *P. aeruginosa* infections include some β-lactams, such as carbenicillin, ticarcillin, third-generation cephalosporins, the synthetic monocyclic β- lactam aztreonam, carbapenems, the aminoglycosides, and the quinolones. Pseudomonas aeruginosa and other fluorescent Pseudomonas species are in general resistant to β-lactams (Garrity *et al.,* 2006). For this reason, current legislation in several countries demands that bottled water products test free of *P. aeruginosa* (Morais *et al.,* 1997). The lack of robust identification tools for these organisms can lead to the misidentification of non-pathogenic Pseudomonas spp. as species; potentially forcing costly and unnecessary food product recalls (Delphine *et al.,* 2008).

Various phenotypic and molecular methods have been developed and used for sub typing bacterial isolates. Phenotypic sub typing methods as biochemical characterization (biotyping), Molecular sub typing methods as polymerase chain reaction (PCR).

The current study was designed to investigate the following points:

1. Isolation and identification of *Pseudomonas aeruginosa* in raw and pasterulized milk.

2. Serological identification of isolated *Pseudomonas aeruginosa* strains

3. Characterization of identified stain by pathogenicity test and antibiotic sensitivity test.

4. Molecular Characterization of identified stain by PCR.

MATERIALS and METHODS

1- Sampling:
A total of 200 samples of raw and pasteurized milk (100 of each) were collected from some super markets in Qaluobia Governorate. Each sample was collected in sterial bags to minimize the possibility of contamination and send to laboratory with minimum of delay.

2- Isolation of *P.aeruginosa:*
According to I. C.M.S.F"1998 one ml from each sample was plated in duplicate using Pseudomonas agar base with 0.1% cetramide, nutrient agar and MacConkey agar and incubated at 37°C for 24-48 h. under aseptic condition.

3- Identification of *P.aeruginosa:*
The suspected colonies were purified and identified biochemically according to Quinn *et al.* (2002).

4- Serological identification:
P. aeruginosa isolated were serogrouped according to Homma (1982) using antisera obtained from Denka Seiken Co .Ltd, Tokyo, Japan.

5- Detection of P.aeruginosa enzymes:
A-Detection of haemolysin:
According to Dennis *et al.* (1980), the isolates were streaked onto blood agar plates and incubated at 37°C for 24 hours. Haemolysis was determined by existence of clear zone around the colony.

B- Detection of phospholipase C (lecithinase):
According to Vanderzant and Splittstoesser (1992), P.aeruginosa isolates were inoculated on the surface of egg yolk agar medium and incubated at 37°C for 24-48 hours. Positive result was indicated by appearance of colonies surrounded by zone of opacity.

C- Detection of caseinase:
According to (Cappuccino and Sherman, 1999), P.aeruginosa isolates were inoculated on the surface of milk agar medium and incubated at 37°C for 24 hours. Positive result was indicated by zone of clearing beneath and around the growth due to clear and cut reactions developing in 24-48 hours.

D- Detection of gelatinase:
According to (Vanderzant and Splittstoesser 1992) nutrient gelatin tube was stabbed with P.aeruginosa culture and incubated at 37°C for 7 days; liquefaction was noted at intervals by removing the tube from incubator and holding it at 4°C for 30 minutes before reading the result. Positive result was noted if gelatin was still in a liquid form.

5- Pathogenicity in laboratory animals:
According to (Ibrahim 2009). (mouse lethality test) 168 albino white mice with average weight of about 18-20 g. and aged 28-30 days old were used to investigate the pathogenicity of 20 K isolates, 22 H isolates and 14 A isolates of *P. areuginosa.* Three mice were used for examination of each isolates. All mice were examined bacteriologically to ensure their freedom from pathogens. The mice were inoculated I.P.with 0.1 ml of 5×10^8 C.F.U./mouse of the tested isolate. Mice of all isolates of the same serogroup were kept together. Last 3 mice were kept under observation for 7-10days, the numbers of dead mice were recorded and re-isolation of the inoculated isolates was done.

6- Drug susceptibility testing:
By using disc diffusion standard technique according to National Committee for Clinical Laboratory Standards (2002) using the following (Oxoide) discs: ciprofloxacin (5ug), enrofloxacin (5ug), norfloxacin (5ug), lomefloxacin (5ug) tobromycin (10ug), ceftriaxone (10ug), gentamycin (10ug), streptomycine (10ug), tetracycline (30ug), erythromycine (15ug) (trimethoprim (25ug) doxycen (20ug), nalidixic acid (30ug).

7- Characterization of isolated P.aeruginosa by polymerase chain reaction (PCR):
DNA Extraction Genomic DNA was prepared from isolates according to (Liu *et al.,* 2002). Ten ml overnight cultures were prepared in broth media from fresh single colony. Cells were harvested in a centrifuge for 5 min at 6000 rpm. then suspended in 200 µl 1xTE buffer (pH8),then 30 mg/ml lysozyme to the cell Suspensions was added and incubated for 2 hours at 37°C. After the incubation, 370 µl, 1x TE (pH 8) containing Proteinase K (1mg/ml) was added for 1 hour then 30 µl, 10% SDS were added. The samples were then incubated for 1 h at 37°C. then phenol Chloroform extraction was performed using one equal volume of phenol /chloroform/isoamyl alcohol (24/24/1) for 30 minutes and then, samples were centrifuged for 5 min at 6000 rpm.then the aqueous phase was transferred into a clean eppendorf tube and the genomic DNA was precipitated by the addition of cold isopropanol (one equal volume) after addition 10% of the volume by ammonium acetate and. Finally, the pellet was dissolved in TE buffer. The extracted DNA was performed was stored at −20°C until use.

Primer design. Relevant 16S rDNA sequences available in the GenBank database were species-specific primers were designed, (Anzai *et al.,* 2000) as in Table (1).

PCR amplification of targeted DNA was carried out in 25-µl reaction volumes, each containing 2 mM $MgCl_2$, 50 mM Trizma (pH 8.3; Sigma, St. Louis, Mo.), 250 µM (each) deoxynucleoside triphosphates (Promega, Madison, Wis.), 0.4 µM (each) primer, 1 U of *Taq* polymerase (Invitrogen, Carlsbad, Calif.), and 2 µl of whole-cell bacterial lysate, and adjusted to 25 µl by the addition of high-performance liquid chromatography-grade H_2O. Amplification was carried out in a Rapid Cycler (Idaho Technology Inc., Salt Lake City, Utah) thermocontroller. After an initial denaturization for 2 min at 95°C, 25 cycles were completed, each consisting of 20 s at 94°C, 20 s at the appropriate annealing temperature (Table 1), and 40 s at 72°C. A final extension of 1 min at 72°C was applied. With this program, the total time for amplification of target DNA was approximately 45 min. PCR products amplifications were detected on standard (# SM0323) Fermentas was used as a marker, and visualized under UV light.

Table 1: Oligonucleotide primers used for amplification of the *P. aeruginosa* 16S rDNA-based primer sets.

Primer	Sequence (5'-3')	Target	Annealing temp (°C)	Location	Product size (bp)
PA-SS-F	GGGGGATCTTCGGACCTCA	*P. aeruginosa* 16 s	58	189-206	956
PA-SS-R	TCCTTAGAGTGCCCACCCG			1124-1144	

RESULTS

Table 2: The prevalence of P. *aeruginosa* isolated from milk samples

Types of samples	No of examined sample	positive sample	
		No	%
Raw milk	100	40	40
Pasteurized milk	100	16	16

Table 3: Frequency distribuation of Serogrouping of *P.aeruginosa* isolated from examined samples

Type of sample	No of isolated strain	Serogroupes	No	%
Raw milk	40	K	16	40
		H	14	35
		A	10	25
Pasteurized milk	16	K	4	25
		H	8	50
		A	4	25

Table 4: Detection of enzyme of *P. aeruginosa* isolated from pasterilized and raw milk samples

Enzyme	Serogroupe					
	K(20)		H(22)		A(14)	
	No.	%	No.	%	No.	%
Hamemolysine	12	60	12	54.5	6	42.8
Phospholipase	10	50	10	45.5	4	28.5
Caseinase	20	100	22	100	14	100
Gelatinase	18	90	18	81.8	12	85.7

Table 5: Pathogenicity test of *P.aeruginosa* isolated from milk samples

Serogroupes	No.of serogroupes	No.of tested mice	No.of dead mice	%
K	20	60	60	100
H	22	66	66	100
A	14	42	24	57.14
Total	56	168	150	89.28

Table 6: Antibiotic sensitivity test of *P.aeruginosa* isolated from milk samples

Antibiotic disc	Serogroups						Total	
	K(20 strains)		H(22strains)		A(strains)			
	No.	%	No.	%	No.	%	No.	%
Ciprofloxacin	12	60	16	72.72	6	42.85	34	60.7
Enrofloxacin	10	50	16	72.72	4	28.57	30	53.6
Norfloxacin	10	50	16	72.72	4	28.57	30	53.6
Lomefloxacin	10	50	16	72.72	4	28.57	30	53.6
Tobromycin	4	20	10	45.45	0	00.00	14	25.0
Gentamycin	4	20	8	36.36	0	00.00	12	21.4
Ceftriaxone	4	20	6	27.27	0	00.00	10	17.9
Streptomycin	2	10	4	18.18	0	00.00	6	10.7
Erythromycine	0	00	2	9.09	0	00.00	2	3.6
Tetracycline	0	00	2	9.09	0	00.00	2	3.6
Trimethoprim,	0	00	0	0.00	0	00.00	0	0.0
Naldixic acid	0	00	0	0.00	0	00.00	0	0.0
Doxycen	0	00	0	0.00	0	00.00	0	0.0

PCR results:

16s rRNA sequencing

16S rRNA analysis was performed on all the *P.aeruginosa* isolates, initially using species-specific oligonucleotide primers designed by (Spilker *et al.*, 2004). The primer set, based on *P.aeruginosa* conserved regions of the 16s rRNA and designed for the differentiation of *P.aeruginosa* from other *Pseudomonas* species, PCR amplification products of 956 bp were obtained for the *P. aeruginosa* isolates compare with control positive (native organ AHRI serology unit) as

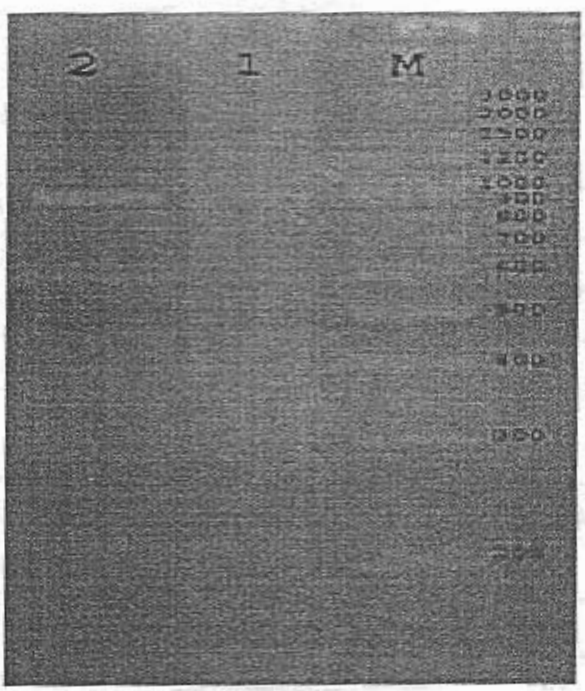

Fig (1): Agarose gel electrophoresis 1% at 80 Volt /cm of whole genomic DNA of *P. aeruginosa* isolates Lane 1: *P aregunosa* appeared at 956 bp fragment, lane 2 the positive samples (native organ AHRI Serology Unit) and M: marker 100 bp standard (#SM0323) Fermentas.

DISCUSSION

Contamination of dairy product with Pseudomonas microorganisms is of a particular concern for the dairy industry as dairy products are distributed at temperatures permissive for the growth of these organisms (Ray, 2004).

Psychotropic bacteria typically enter processed dairy products through post pasteurization contamination in the milk processing plant (Khan *et al.,* 2008). Bacterial spoilage ensues when growth conditions during refrigerated storage allow psychotrophic microbes to increase in number and to become the dominant microflora (Dogan and Boor, 2003).

It was evident from Table (2) that the *P.aeruginosa* was recovered from raw and pasteurized milk with an incidence of 40 % and 16 %, respectively. Nearly similar results were reported by Delphine *et al.* (2008); Laura and Mauro (2007) and Parkash *et al.* (2007).

To produce the best quality milk and to achieve all the nutritious benefits of it, the highest quality raw milk must be obtained. The conditions of heat treatment used for pasteurization depend on the final product; lower temperatures are used for refrigerated products and higher heat treatments are used for products stored at room temperature (United States Code of Federal Regulation [USCFR], 2006.

In Table (3) *Pseudomonas aeruginosa* isolated from milk samples belonged to 3 serogroups K, H, A. The predominant serogroupe was H (22 isolates) followed by K (20 isolates) then A (14 isolates), nearly similar results was recorded by Laura and Mauro (2007).

Most of *P.aeruginosa* infections are both invasive and toxigenic as it produce variety of structure and products including outer membrane protein, pilli, enzymes and toxins, which are considered as a major cause of its virulence (Ibrahim, 2009).

In Table (4) there were significant differences among virulence markers of *P. aeruginosa* in relation to their serogroupes. Serogroupe K was highly virulent followed by H then A.

Serogroupe K produce Hameolysine, Phospholipase, Caseinase and Gelatinase in percent of 60, 50,100 and 90, respectively, while serogroupe H produce it in percent of 54.5, 45.5, 100 and 81.8, respectively, in the same while serogroupe A produce it in the percent 42.8, 28.5, 100 and 85.7, respectively, Nearly similar results were reported by Braun and Fehlhaber, (2002) and Geetha and Prasad (2001). Extracellular enzymes (mainly lipases and proteases) that are secreted by these organisms are known to cause spoilage of milk and dairy products, leading to important economic losses (De Jonghe *et al.,* 2010). Lipases degrade the milk fat, causing rancid, soapy, and occasional bitter off-flavors through the formation of medium-chain fatty acids. Proteases that degrade casein cause a gray color, bitter off-flavors, and gelation of ultra high-temperature (UHT) products (Datta and Deeth, 2001). Psychrotolerant bacteria have become more important for the shelf life of heat-treated dairy products because of the development of these bacteria during prolonged refrigerated storage of raw milk on the farm and at the dairy plant. In an effort to reduce the total aerobic plate count of raw milk, a lower storage temperature (1 to 4°C) is upheld; leading to the perception that raw milk could be stored for a longer period before further processing. However, the combination of a longer storage time and a lower temperature creates a selective advantage for psychrotolerant bacteria, especially Pseudomonas members that enter raw milk via biofilms in the milk tanks, contaminated water, and soil (Simões *et al.,* 2009). These pseudomonads are able to outgrow other bacteria, such as members of the *Aeromonas, Listeria, Staphylococcus, and Enterococcus* genera and the family Enterobacteriaceae, thus becoming the predominant microbes in raw milk (Lafarge *et al.,* 2004), constituting up to 70 to 90% of the psychrotrophic raw milk microbes (Coorevits *et al.,* 2008). Even though they are easily inactivated through pasteurization or UHT treatment, their heat-resistant enzymes persist upon processing of the milk (Chen *et al.,* 2003).

In Table 5, Pathogenicity test of *P.aeruginosa* revealed that K and H strains gave 100% mortalities in mice, while strain A gave lower mortalities 57.14%. (Ibrahim, 2009) isolated the same strains and found nearly the same result of pathogeniciy in mice.

In Table 6, *P.aeruginosa* varied in their resistance to different antibacterial agents, ciprofloxacin was the most effective drug against *P.aeruginosa* (60.7%), followed by enrofloxacin, norfloxacin and lomefloxacin (53.6%). *P.aeruginosa* was low in susceptibility to tobromycin (25.0%), gentamycin (21.4%) ceftriaxone (17.9%) streptomycin (10.7%) erythromycin (3.6%) tetracycline (3.6%). The bacterium was completely resistant to trimethoprim, naldixic acid and doxycen. Similar results were recorded by Arslan *et al.* (2011), while Amany *et al.* (2006) and Crespo *et al.* (2004) found that *P.aeruginosa* isolated from some clinical and environmental samples was sensitive to gentamicin and ciprofloxacin, this might be due variation in the usage of antibiotics in different geographical areas. *P.aeruginosa* is a clinically significant pathogen characterized by intrinsic resistance to number of antimicrobial agents, moreover problem with the development of resistance to agent generally exhibiting potent antibacterial activity against this organism are encountered with increasing frequency

and cross resistance to chemically unrelated antibiotics (Muramatsu *et al.*, 2005).

Two primer pairs were used in this study; the pair PA16S-F and PA16S-R which was specific to *P. aeruginosa.* These primers targeted the variable regions in the 16S rRNA gene. PCR assays employing this primer pair produced DNA products of the predicted size (Figure: 1). 16S rRNA gene sequence offered a useful method for the identification of bacteria. It had long been used as a taxonomic method in determining the phylogenies of bacterial species (Drancourt *et al.*, 2000). Genomic DNA This results agree with Khulod *et al.* (2012) and Theodore *et al.* (2004) they recorded the band at 956 bp.16s rRNA sequencing is therefore now considered to be the hierarchy in phenotypic identification (Woese, 1987). So 16s rRNA sequencing was performed on all the "*P.aeruginosa*" isolates for definitive identification, as shown. 16s rRNA genes are conserved among all organisms however possess various unique species regions that allow bacterial identification (Gobel *et al.*, 1987). However, the advancement in 16s rRNA analysis has resulted in the development of rapid diagnostic techniques for the identification of *P.aeruginosa* (O'Callaghan *et al.*, 1994; LiPuma *et al.*, 1999; Spilker *et al.*, 2004).

PCR used this method in genotypic characterization of *Pseudomonas* strains isolated from bulk tank milk and act as a rapid and accurate method for typing *Pseudomonas spp* (Wang and Jayarao, 2001).

In this study, the presence of Pseudomonas aeruginosa after pasteurization may be due to insufficient pasteurization or the post contamination by this genus and the temperature which milk and dairy products are exposed influences the type of microorganisms that will grow in them.

As dairy equipment and utensils constitute the major source of many types of psychrotrophics in milk, so special attention should be considered in their cleaning and sanitation to produce milk of low bacterial count or even completely free of psychrotrophics bacteria. The previous information indicated that psychrotrophics are still inevitable because they are widely distributed in nature, withstand sanitizers and can liberate heat stable enzyme causing spoilage of food and some of them considered as food borne pathogens. This knowledge increase attention toward the way by which the restriction of these microorganisms must be done, we can concluded also that PCR act as a rapid and accurate method for typing *Pseudomonas spp.*

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تحديد الخصائص الجزيئيه لميكروب السيدومونس اريجينوزا المعزول من اللبن

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الهدف من هذا البحث هو دراسه مدى انتشار ميكروب السيدمونس ايرجنوزا فى الالبان ودراسه بعض خصائصه تم فحص عدد ٢٠٠عينه من كل من اللبن الخام والمبستر المجمع من بعض السوبر ماركت الموجودة بالقليوبية. واسفرت النتائج عن وجود الميكروب 40% و 16% فى عينات اللبن الخام والمبستر على التوالى. تم تصنيف الميكروب سيرولوجيا وقد تبعت العترات ثلاث مجموعات سيرولوجيه وهى (A,H and K) بنسبة ٢٥% و ٣٥% و ٤٠% من اللبن الخام على التوالى وبنسبة ٢٥% و ٥٠% و ٢٥% من اللبن المبستر على التوالى تم اجراء اختبارات الضراوة للسيدمونس اريجينوزا وهى اختبارات الهيموليزين الفوسفوليبيز الكازينيز الجيلاتينيز وكانت نتائج العترة K ٦٠و٥٠و١٠٠و٩٠و٨٠ % على التوالى بينما كانت العترة H بنسبة ٥٤.٤ و٤٥.٥و١٠٠و٨١و٨ % على التوالى وكانت العترة A 42.8,28.5,100,85.7% على التوالى 57.14%، 100، 100 بنسبة A,H, K بالنسبه لقدره الميكروب على احداث المرض فكانت على التوالى. اثبتت اختبارات الحساسيه للمضادات الحيويه ان السيبروفلوكساسين هو الاكثر تاثيرا على الميكروب بنسبه 60.7% يليه واللوموفلوكساسين والنورفلوكساسين الانروفلوكساسين بنسبه 53.6%.وكان الميكروب اقل تاثرا التوبروميسين بنسبه 25% يليه% والجنتاميسين بنسبه % 21.4 ثم والسيفتركسيون بنسبه % 17.9 ثم الستروبتوميسين بنسبه 10.7% ويليه الايرثرومايسين والتتراسيكلين بنسبه 3.6% وكانت العترات مقاومه تماما للترامبثوبريم وحمض النالديكسيك والدوكسيسين. لتعريف السيدوموناس اريجينوزا باستخدام الحامض النووى باستخدام البريمرخاص بجين S١٦ وكانت النتيجة اكثر سرعة وحساسية لتميز السيدوموناس اريجينوزا عند ٩٥٦.