Diyala University

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Detection of staph aureus in milk of cows in diyala city

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الاهداء

الى الهادي البشير والسراج المنير الذي كان كلامه تشريعاً بعد كتاب الله عز وجل قدوتنا وامامنا محمد (صلى الله عليه وسلم)

الى العلماء العاملين والوعاظ المرشدين الذين افنوا حياتهم في خدمة هذا الدين .....

والى من سلك طريقاً يلتمس به علماً ليرفع به لواء الاسلام .....

الى والدي الكريم ووالدتي الرؤوم .....

الى إخوتي المؤمنين واخواتي المؤمنات .....

إلى اساتذتي الافاضل الاجلاء .....

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Abstract

Staphylococcus aureus most important species of staphylococcus Isiolated from the milk of normal appearantly cow,50 samples are collected from diffrent regions of diyala shows the present of staphylococcus aureus in most areas while some areas shows not .the prevelance of present of staphylococcus aureus is diffrant from one region to another region acording to the results of the research .the samples were cultured either in selective media (menitol ager) or non selective media(blood and macconkey agar)then the bacteria were isolated ,in selective media it allredy grow while in non selective media the bacteria most be pured and stained with gram stain then identified the staphylococcus aureus which are gram positive bacteria cluster like shape either single or colony .7 samples from 50 samples shows positive results while 43 samples showes nigative results . the percentage of posative results was 14% while negative results was 86%

INTRODUCTION

Staphylococcus aureus is an important food borne pathogen and causes a mild skin infection to more severe diseases, such as pneumonia and septicemia [1]. *Staphylococcus aureus* is a major causative pathogen of clinical and subclinical mastitis [2]. Milk has been reported as a common food that may cause staphylococcal poisoning [3]. The update studies of epidemiology of *Staph. aureus* may help in prevention and control strategies. Isolation of *Staph. aureus* from milk was previously studied in [4] diyala city[5]. Enteropathogenic *Staph. aureus* in milkshould be regarded as a part of the risk analysis of milk and milk products [6]. The importance of contaminated surfaces in relation to potential transmission of pathogensto food is apparent in food processing, catering anddomestic environments [7]. One factor for transmission of a microorganism from a person to the environmentand then to another person is the ability of that microbe to survive on that enviromental surfaces[8].

REVEIEW:-

Staphylococcus aureus

Staphylococcus aureus is a bacterium that causes staphylococcal food poisoning, a form of gastroenteritis with rapid onset of symptoms. *S. aureus* is commonly found in the
environment (soil, water and air) and is also found in the nose and on the skin of humans.

Description of the organism

S. aureus is a Gram-positive, non-spore forming spherical bacterium that belongs to the
Staphylococcus genus. The *Staphylococcus* genus is subdivided into 32 species and
subspecies. *S. aureus* produces staphylococcal enterotoxin (SE) and is responsible for
almost all staphylococcal food poisoning .*S. intermedius*, a *Staphylococcus* species which is commonly associated with dogs and other animals, can also produce SE and has been rarely associated with staphylococcal food poisoning .

Growth and survival characteristics

The growth and survival of *S. aureus* is dependent on a number of environmental factors
such as temperature, water activity (aw), pH, the presence of oxygen and composition of thefood. These physical growth parameters vary for different *S. aureus* strains.The temperature range for growth of *S. aureus* is 7–48°C, with an optimum of 37°C.

S. aureus is resistant to freezing and survives well in food stored below -20°C; however,
viability is reduced at temperatures of -10 to 0°C. *S. aureus* is readily killed during
pasteurisation or cooking. Growth of *S. aureus* occurs over the pH range of 4.0–10.0, withan optimum of 6–7 .*S. aureus* is uniquely resistant to adverse conditions such as

low aw, high salt content andosmotic stress. In response to low aw, several compounds accumulate in the bacterial cell,which lowers the intracellular aw to match the external aw.As such, most *S. aureus* strains can grow over a aw range of 0.83 to >0.99
S. aureus is a poor competitor, but its ability to grow under osmotic and pH stress means
that it is capable of thriving in a wide variety of foods, including cured meats that do not
support the growth of other foodborne pathogens .(9)

Sources of Infection and epidmeology:

The lactating cow's udder is considered the major reservoir of infection for *S. aureus* IMI.
While *S. aureus* can be isolated from environmental sources on farms, the cow's environment is
not considered the major reservoir for infection. The major mode of transmission for *S. aureus*
mastitis is from cow-to-cow via fomites, such as milking unit liners, at the time of milking. The replacement heifer can be a significant source of *S. aureus* IMI. In some areas of the UnitedStates, for example, the prevalence of *S. aureus* IMI in pre-partum heifers exceeds 30%. Areport from Louisiana recorded a prevalence of 37% (Trinidad et al., 1990) and work by the author in Missouri has recorded a prevalence of approximately 30% (Middleton, unpublished data), while heifers in Northern areas of the country seem to have a lower prevalence of prepartum *S. aureus* IMI (Fox et al., 1995). The heifer's mammary gland may become infected as early as six months of age and she may harbor these infections throughout the first lactation. heifers are most susceptible to infection during the lasttrimester of gestation.(10)

How do *Staph. aureus* infections move from cow to cow?

Staph infections are usually transferred from infected cows to noninfected cows during milking via contaminated teat cup liners, milkers' hands, and common wash towels or rags. Flies have also been implicated in the transfer of *Staph. aureus* from one animal to another.

What kind of mastitis problems can *Staph. aureus* cause?

Because of its toxin (poison) production, *Staph. aureus* can cause mastitis problems

ranging from non-clinical infections to clinical or gangrenous infections that may kill the cow. Once *Staph. aureus* gets into the mammary gland, it will invade deep into secretory cells and ductal tissue. Staph infections produce scar tissue and can cause small abscesses in the udder. The small abscesses can break open at anytime causing a re-appearance of clinical signs or elevated somatic cell counts. Scar tissue and micro-abscesses may permanently limit an infected quarter's ability to produce milk and to respond to treatment efforts.(11)

Members of this genus are catalase-positive and oxidase-negative, distinguishing

them from the genus streptococci, which are catalase-negative, and have a different cell wall composition to staphylococci. Staphylococci are tolerant to high concentrations of salt and show resistance to heat. Pathogenic staphylococci are commonly identified by their ability to produce coagulase, and thus clot blood and. This distinguishes the coagulase positive strains, *S. aureus* (a human pathogen), and *S. intermedius* and *S. hyicus* (two animal pathogens),from the other staphylococcal species such as *S.epidermidis*, that are coagulase-negative (CoNS).(12)

Pathogenesis:-

Intramammary infection occurs by *S. aureus* entering the teat orifice, breaching the streak canal,and entering the mammary gland. Teat skin condition and damage to streak canal keratin can impact the likelihood of IMI with chapped or injured teats being more likely to develop IMI.Once in the mammary gland, *S. aureus* adheres to mammary epithelial cells decreasing the

likelihood of the bacterium being washed out of the gland by milking. The primary defense against IMI is phagocytosis (ingestion) of *S. aureus* by neutrophils, the primary somatic cell involved in clearance of pathogens from the mammary gland. However, neutrophil phagocytic function is impeded in the presence of milk, and *S. aureus* produces a number of antiphagocytic
factors, such as protein A, capsule and pseudocapsule, that decrease its likelihood of
phagocytosis by neutrophils. Furthermore, the concentrations of opsonizing antibodies and complement, factors which facilitate uptake of *S. aureus* by neutrophils, are low in milk further impeding *S. aureus* clearance Once established in the gland, *S. aureus* IMI can lead to ulceration and erosion of the lactiferous sinus and ducts, infiltration of the parenchymal tissue with inflammatory cells, and ultimately damage to the mammary secretory epithelial cells leading to occlusion of the ducts and alveoli which can trap *S. aureus*. These trapped bacteria can become a nidus for infection of other portions of the gland or lead to granulomas and microabscesses .Tissue damage in the mammary gland is further compounded by toxins and extracellular enzymes produced by *S. aureus*, including α, β, γ, and δ toxins, toxic shock syndrome toxin (TSST-1), enterotoxins, nuclease, coagulase, catalase, hyalurodinase, phosphatase, lipase, staphylokinase, and proteases. Toxins can have direct effects on tissue or lead to an uncontrolled host inflammatory response by acting as superantigens. The involvement of enzymes in mastitis pathogenesis is not well understood, but it is hypothesized that they may facilitate pathogen adaptation to growth in milk (13).

50 samples of raw milk and 20 samples of pasteurized milk were analyzed from 10 municipalities of this region (Cabaceiras do Paraguaçu, Cachoeira, Conceição do Almeida, Cruz das Almas, Dom Macedo Costa, Maragogipe, São Sebastião do Passé, Saubara, Santo Amaro and Santo Antônio de Jesus). The *Staphylococcus aureus* was isolated in Baird Parker agar where typical

and atypical colonies were selected and submitted to coagulase and complementary tests. Out of 50 samples of raw milk studied, 34 showed contamination by *Staphylococcus aureus*, corresponding to 68% of the samples being contaminated. In the pasteurized milk, 6 samples were contaminated with this microorganism, corresponding to 30% of the 20 samples. The presence of this pathogenic microorganism indicates a potential health hazard to those who consume milk from this region.(14).

. The effects of sampling time and sample handling were examined in an attempt to improve the accuracy of detection of *S. aureus*. Premilking and postmilking milk samples were collected from 55 lactating quarters with subclinical *S. aureus* infection. Each sample was divided into 2 aliquots; one of which was cultured fresh, the other was frozen at -20°C for 14 days before being cultured. Analysis of variance was used to determine the effect of sampling time (premilking vs postmilking) and sample handling (fresh vs frozen) on the detection of *S. aureus*, as measured by the mean category for colony-forming units per millilitre (cfu/mL). A stratified analysis was required, due to interaction between sampling time and sample handling. Only a fresh postmilking sample was inferior, yielding a lower mean category for cfu/mL ($P < 0.05$). The ability to detect *S. aureus* in quarters with subclinical intramammary infection, as measured by the mean category of cfu/mL, was maximized in fresh or frozen premilking samples and in frozen postmilking samples (15).

Surveys conducted in Senegal have shown a strong association of staphylococci with subclinical mastitis in dairy cows. This study aimed to characterise *Staphylococcus aureus* strains identified in the dairy farms in Dakar. Of a total of 244 Staphylococcus spp isolates col ected from 135 lactang cows with subclinical mastitis at six dairy herds in peri-urban region

of Dakar, 109 S. aureus strains were isolated and idenfied using phenotypic methods. Using PCR, genes of thermonuclease and major capsular type 5 and 8 angens were idenfied respecvely in 98.17%, 93.58% and 1.84% of S. aureus strains. Similarly, the spa gene was found in 68.81% of S. aureus strains, luk D (62.39%) and luk S (0.92%). Among the virulence genes sought, spa, Luk S and Luk D genes were found respecvely in 68.81%, 62.39% and 0.92% strains of S. aureus. However, the lukF, lukM and tst1 genes were absent in al isolated S. aureus. Among the six enterotoxins analysed, none of the S. aureus harboured the genes sea, sec and seh. Only the seb, sei and sej were found respecvely at rates of 2.75%, 3.67% and 26.6%. Final y, the gene for resistance to methicil in was found in 2.75% of the strains isolated. The analysis of the association between virulence genes and the presence of subclinical mastitis showed the importance of genes Spa lukD and SEJ. Antibiotic resistance testing revealed a good sensitivity of S. aureus to most of the antibiotics tested. This study showed the low virulence of the S aureus strains isolated.(16)

Materials and methods:

1-mannitole ager

2-macconkey ager

3- gram stain

4-microscope

5-Aseptic contaner

6-burner

7-plate redsh

8-autoclave

9-microbalance

10-loope

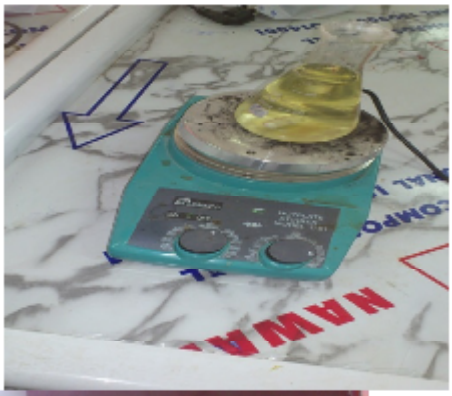
11-hote plate shaker

12-incubation

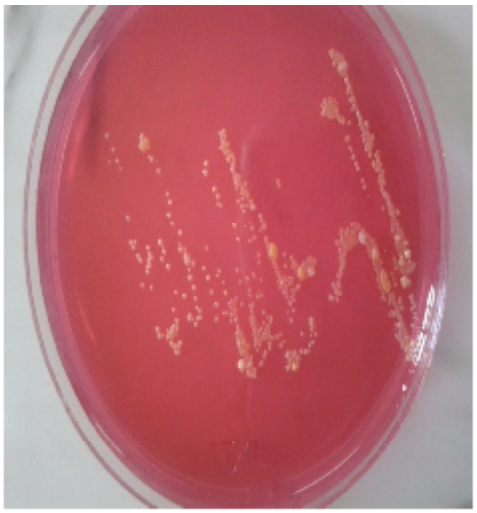
13-cloves and mask

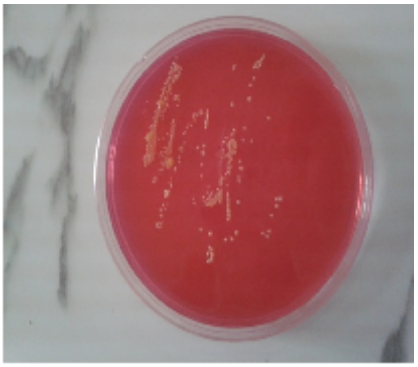










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50 samples (cows) are collected from diffrent area of diyala city ,the udder of the sampled cows were apparently normal. Each milk sample was collected in a sterile screw cap bottle aseptically from each mammary gland after washing with water and cleaning the teats with cotton soaked in 70% ethanol and previous discard of the first 3 streams of milk. The samples are taken to the laboratory for bacteriological analysis. Identification of suspected *Staph. aureus* colonies was carried out.

In the case of selective media(manitol agar) ,firstly agitate the sample to hemogenized the content then near the burner open the sample and socked the loope in the screw cap bottle then cultivate in to the selective media (manitol agar) ,place the selective media in incubation(37c) for 24hours ,the bacteria allrady grow if present after this period.

in the case of non selective media (blood and macconkey agar) the first steps is similar to that in selective media until place the media in the incubation for 48hours in tempareture 37c ,after that the media show growth of diffrent bacteria ,therfore,the bacteria most be pured in new cultuer media by taking by using loope the bacteria from the end of growing bacteria(pure) ,then the new media cultvate in incubation in the same tempareture for 24hours ,the result is pure bacterial growth in the media .

after that the bacteria most be stained with gram stain to identefied the staphylococcus aureus under microscope which are gram positive bacteria,grap like clusters

RESULTS:

The samples are collected from diffrent area of diyala city ,these samples taken from normal appaerantly cows and the number of it was 50 and shows the results as following:

total	السالب	الموجب	عدد النماذج	اسم المنطقة
30%	7	3	10	خان بني سعد
10%	9	1	10	العظيم
20%	8	2	10	المنصورية
0%	10	0	10	الوجيهية
10%	9	1	10	الخالص
70%	86	14	50	Total

posative results of research 14% ,negative results 86%

DISCUSSION:

The aim of this research is to find any spread of staph.aureus organism in milk of cows in baquba city .by taking 50 sample of milk randomly from different area of baquba city ,from the result we discovered 7 sample positive and others sample negative this refer the persent of contamination of milk with staph aureus organism which causes many disease to the animal And humen by consumption the product of animal specially milk And damy product may be contamination .we see from result 30% of contamination in kan bany saad this percentage is very high And may causes desease to Animal may be clinical And sub clinical signs find .the total result posative Are 14% this number is significant to treat the Animal.

Recommendations:

1-We recommend to aware the farmer of how dangerous is infection of staph .aureus on the animal and also on its production of milk and meat.

2-Teated of farm animal periodically from the used against staph aureus and in the regular program.

3-Periodical check from veterinarian randomly on cows and diagnosis the any infection by staph and heal it to get rid of organism

4-To get rid of average host this is the main cause to spread the organism among animals.

5-Provid good health care by building health houses And aseptic instrument of milking And milkers

الخلاصة:-

المكورات العنقودية من اهم انواع المكورات التي تم عزلها من حليب الابقار السليمة ظاهريا ،50عينة تم جمعها من مناطق مختلفة لمحافظة ديالى اظهرت وجود هذه المكورات العنقودية في اغلب المناطق بينما بعض المناطق لاتظهر وجود هذه المكورات العنقودية .تختلف درجة تفضيل وجود هذه المكورات العنقودية من منطقة الى اخرى طبقا لنتائج البحث.العينات تم زرعها في اوساط خاصة لهذه المكورات واوساط غير خاصة وبعد ذلك تم عزل البكتريا،ففي الاوساط الخاصة تنمو هذه البكتريا حيث لاتنمو انواع غيرها ،اما في الاوساط الغير خاصة تنمو عدة انواع من البكتريا لذلك يجب عزلها من البقية وذلك عن طريق زراعتها بصورة نقية وبعد ذلك يتم صبغها بصبغة الغرام ويتم التعرف عليها بواسطة المجهر حيث تظهر عنقودية السكل موجبة الصبغة .7عينات من اصل 50 عينة تظهر نتائج موجبة بينما 43عينة تظهر نتائج سالبة ،نسبة النتائج الموجبة 14% بينما نسبة النتائج السالبة 86%.

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