Republic of Iraq Ministry of higher education and scientific research Diyala university Veterinary medicine college

EVALUATION OF CARCASS WITH STAPHYLOCOCCUS AUREUS

A seminar submitted to the council of the veterinary medicine at university of Diyala in partial fulfillment of requirement for the degree of bachelor in surgary and veterinary medicine

By

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ABSTRACT

This study was conducted to isolate & identify of Staphylo . aureus on specific culture media from (50) samples of carcass form five different area of Diyala governorate.(Alamin, Baquba Algededa, AL catwon, AL tahrer & Almrfuq) most of samples which are collected from the Baquba Al gadeda area found that (4) samples are (+ve) while (6) samples are (-ve)' but in Al tahreer area (2) samples was (+ve) and (8) samples are (-ve) while in Alameen area (3) samples was (+ve) and (7) samples are (-ve) and in Al mafrage area found that only(1) samples is (+ve) and (9) samples are (-ve) but the samples which are collected from Al catwon area found that are from staphylococcus aureus and the result are (-ve). This result may be releated to the contamination of Carcass may be during slauthring of the animal or instrument that are used in slauthring or cutting of meat and may be the contamination of the carcass introduce from personal which are work in slautring house or in market butcher

INTRODUCTION

The carcass was contaminate by microbial , parasite , viruses , fungus and other agent as it consider a good media for growth of both pathogenic & nonpathogenic microorganisms as it contain main food substance for different bacterial $^{(1)}$.

This studies referred to the presence of pathogenic microorganisms in meat especially that is cause food poisoning for human such as Campylobacter ,Staph.aureus&Salmonella^{(2).}

There are many factors that contribute to spreading of pathogenic microorganisms in farm , methods of collection of meat , environment , person whose work in sluaghtring & house or market butcher⁽³⁾.

THE AIMS :

- 1- To evaluate the hygienic potential of meat used for human consumption in diyala .
- 2- To determine the incidences of Staph.aureus in meat.
- 3- To identify the source of contamination in meat .

Literature review

Staphy. Aureus

Characterized :

Gram stain of S.aureus Cells which typically occur more in clusters than chains , and the cells take up gram stain well S.aureus is a facultativelyanaerobc , Gram positive coccus , which appears as grape – like clusters when viewed through amicroscope , and has large , round , golden – yellow colonies , often with hemolysis , when grown on blood agar plates ⁽⁴⁾ the Golden appearance is the etymological root of the bacterium's name ; aureus means golden in Latin .

S.aureus is catalase - positive (meaning it can produce the enzyme catalase), so is able to convert hydrogen peroxide to water and oxygen, which makes the catalase test useful to distinguish staphylococci from enterococci and streptococci . A small percentage of S.aureus can be differentiated from most other staphylococci by the coagulates test: S.aureus is primarily coagulates - positive (meaning it can produce the enzyme coagulase) that causes clot formation whereas most other staphylococcus species are coagulase – negative . ⁽⁵⁾ However, while the majority of S.aureus are coagulase – positive, some may be a typical in that they do not produce coagulase (the most common organism in patients with nosocomial bacteremia (6) coagulase – negative staphylococcus is . Incorrect identification of an isolate can impact implementation of effective treatment and / or control measure (7).

Toxic shock syndrome and S.aureus food poisoning

Some strains of S.aureus , which produce the exotoxin TSST-1 are causative agents of toxic shock syndrome . Some strains of S.aureus gastroenteritis . The gastroenteritis is self – limiting , with the person recovering in (8-24) hours(3) . Symptoms include nausea , vomiting , diarrhea , and major abdominal pain. Lack of antibody to TSST – plays a part in the path in the pathogenesis of toxic shock syndrome .(1)

Virulence factors

Toxins

Depending on the strain, S.aureus is capable of secreting several exotoxins, which can be categorized into three groups. Many of these toxins are associated with specific diseases .(7)

Super antigens

(PTSAgs) have superantigen activities that induce toxic shock syndrome (TSS). This group included the toxin TSS-1, which causes TSS associated with tampon use . This is characterized by fever , erythematous rash , hypotension , shock , multiple organ failure , and skin desquamation . The staphylococcal enterotoxins , which cause a form of food poisoning characterized by vomiting and diarrhea one to six hours after ingestion of the toxin , are included in this group . Exfoliative toxins EF toxins are implicated in the disease staphylococcal scalded-skin syndrome (SSSS) , which occurs most commonly in infants and young children . It also may occur as epidemics in hospital nurseries . The protease activity of the exfoliative toxins causes peeling of the skin observed with SSSS⁽⁸⁾.

Other toxins

Staphylococcal toxins that act on cell membrane include alpha toxin, beta toxin, delta toxin, and several bicomponent toxins. The bicomponent toxin panton-Valentine leukocidin (PVL) is associated with severe necrotizing pneumonia in children. The genes encoding the components of PVL are encoded on a bacteriophage found in community associated methicillin resistant S.aureus (MRSA) strains .(7)

Protein A

Protein A is anchored to staphylococcal peptidoglycan pentaglycine bridges (chains of five glycine residues) by the transpeptidasesortase A ⁽⁹⁾ protein A , an IgG-protein , binds to the Fc region of an antibody . In fact , studies involving mutation of genes coding for protein A result in a lowered virulence of S.aureus as measured by survival in blood , which has led to speculation that protein A-contributed virulence requires binding of antibody Fc regions ⁽¹⁰⁾ . Protein A in various recombinant forms has been used for decades to bind and purify a wide range of antibodies by immunoaffinity chromatograph . Transpeptidases , such as the sortases responsible for anchoring factors like protein A to the staphylococcal peptidoglycan , are being studies in hopes of developing new antibiotic to target MRSA infections ⁽¹¹⁾.

Role of pigment in virulence

Some strains of S.aureus are capable of producing staphyloxanthin – a carotenoid pigment that acts as virulence factor. It has an antioxidant action that helps the microbe evade death by reactive oxygen species used by the host immune system. Staphyloxanthin is responsible for its characteristic golden colour. When comping a normal strain was colonies of the two strains were also exposed to human neutrophiles. The mutant colonies quickly succumbed , while many of the pigmented colonies survived. Wounds on mice were inoculated with the two strains . The pigmented strains created lingering abscesses . Wound with the un pigmented strains healed quickly.

These tests suggest the staphyloxanthin may be key to the ability of S.aureus to survive immune system attacks . Drugs designed to inhibit the bacterium's production of the staphyloxanthin may weaken it and renew it's susceptibility to antibiotics . In human cholesterol , a drug developed in the context of cholesterol lowering therapy was shown to block S.aureus pigmentation and disease progression in mouse infection model . (9)

Source and Transmission

The major site where people carry S.aureus is in the nasal passages . Approximately 25% to 30% of the population is colonized ⁽⁵⁾(when bacteria a present , but not causing an infection) with S.aureus , but only about 1% of the population is colonized with MRSA . The main mode of transmission of staph and MRSA is through hands , which may become contamined by contact with colonized or infected individuals or through contact with colonized or infected body sites of the other persons . Contact with devices , items , or environmental surface contaminated with body fluids containing staph or MRSA may also cause infection . Other factors contributing to transmission include close skin to skin contact , crowded conditions , and poor hygiene . (2)

Studies in recent years have demonstrated that food producing animals also carry MRSA . Studies conducted in the U.S. as well as several other countries , including Austria , Canada , China , abelgium , Denmark , France , Italy , South Korea , Taiwan and the Netherlands , have isolated MRSA mainly from pigs . Other animals testing positive for MRSA have included chickens , cattle and dairy cows . In addition , the testing of raw meat samples from slaughter and retail markets has revealed MRSA in several countries . This is likely due to the high use of antibiotics in food animal production . Estimated of the amounts of growth promoting antibiotics used in U.S. in fact , the presence of MRSA in food producing animals has also led to the transmission of MRSA to farmers . (6)

Their families , and veterinarians , resulting in human colonization of even greater concern is the identification of MRSA in retail meats and food products, including pork, beef, and diary products. This has occurred in the U.S. as well as in Austria, China, the Netherlands, Portugal, and South Korea. However, few food borne or food initiated outbreaks have been reported . One food initiated out break in the Netherlands involved the transmission of MRSA from a colonized but healthy hospital dietary worker to a patient through food. The contaminated food (which tested positive for MRAS) was ingested by the patient who was severely immune compromised, and the patient contracted a fatal infection (4). Transmission from that patient to other hospital workers and subsequently to other patients resulted in a major outbreak . In another food related case, a community acquired food borne illness outbreak occurred in Tennessee . In that outbreak , family developed typical food borne illness symptoms after eating food prepared by a commercial food handler who was colonized with MRSA .(1)

MATERIALS AND METHODS



1- Sample collection

The meat samples were collected from diyala area (Alamin

,BaghubaAlgededa,Almafrag,Alcatwon&Altehrer) for each area (10) samples .

Samples were aseptically collected by wipe the carcass with cotton swabs moisture with normal saline & than

Cotton swabs were streaked on selective media (Staphllo 110& Blood agar) then incubated in $37c^{\circ}/24hrs$ under aerobic condition .

(10) samples from	(+)ve to	(-)ve to
each area	Staph.aureus	Staph.aureus
Baquba .Algededa	4	6
Altehrer	2	8
Alamin	3	7
Almafrag	1	9
Alcatwon	Zero	10
Total	10	40

2. Study methodology :

The plates were examined for presence of staph colonies . Isolates supposed to belong to staphylococcus species on the basis of their morphological aspect (creamy , grayish , white or yellow colonies) . (11)

3. Isolation and identification :

Final identification of staphylococci organisms and species assignment were done based on gram staining , catalase test , sugar fermentation and coagulase test , but this tests not working because there are not found their materials .(11)

4. Classical diagnosis



Typical Gram – positive cocci , in clusters , from a sputum sample , Germ stain depending up on the type of infection present , an appropriate specimen is obtained accordingly and sent to the laboratory for definitive identification by using biochemical or enzyme – based tests(1) . A Germ stain is first performed to guide the way , which should show typical Gram – positive bacteria , cocci , in clusters . Second , the isolated is

cultured on mannitol salt agar , which is a selective medium with 7-9% NaCl that allows . S.aurues to grow , producing yellow – colored colonies as a result of mannitol fermentation and subsequent drop in the medium's pH . Furthermore , for differentiation on the species level , catalase (positive for all staphylococcus species) , coagulase (fibrin clot formation , positive for S.aursus) , DNAse (zone of clearance on nutrient agar) , lipase (a yellow color and rancid odor smell) , and phosphatase (a pink color) tests are all done . For staphylococcal food poisoning , phage typing can be performed to determine whether the staphylococci recovered from the food were the source of infection . (3)

Rapid diagnosis and typing



Diagnostic microbiology laboratories and reference laboratories are key for identifying outbreaks and new strain of S.aureus. recent genetic advance have enable reliable and rapid techniques for the identification and characterization of clinical isolates of S.aureus in real time . These tools support infection control strategies to limit bacterial spread and ensure the appropriate use of antibiotics . Real – time PCR is being increasingly employed in clinical laboratories as a techniques to identifying outbreaks (12).

THE RESULTES

Incidence of Staph.aureus

The result of the present study revealed that 20% of samples collected from(Baghuba Algededa,Alteher,Alamin,Almafrag&,Alcatwon) were contaminated with Staph.aureus according to laboratory test and culture technique .

Table (1) prevalence of Staph. aurous isolated from meat in diyala province .

Area	(+) ve	(-)ve
Baquba.Algededa	(4) 40%	(6) 60%
Altehrer	(2) 20%	(8) 80%
Alamin	(3) 30%	(7) 70%
Almafrag	(1) 10%	(9) 90%
Alcatwon	(0) 0%	(10) 100%
Total = 50	(10) 20%	(80) 40%



CONCLUSIONS

This study do to microbial evaluated of the Carcass with same microorganism specially staphylococcus aureus in different area in Baquba city and also to know the ways that may be caused contamination of the carcass with this microorganism there are different ways that may be staphylococcus aureus transmitted to the carcass principle or specially through workers ,instruments that are used in slaughtering of the animal and also through nasal discharge or bad habit of workers and also un clean slaughtering house.

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