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Isolation and identification *Staphylococcus aureus* from Cows ruminal content

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Abstract

This study was carried out for isolation of *Staphylococcus* aureus and identification it from Cow´s ruminal contant through take of specimens and caltured it on (MSA), the percentage of isolation on this media about 52%, Biochemical tests were done. In biotyping test the percentage reveled in type C about 61.5% while in type A 38.4% .The specimens gave 76% for pigment on Milk agar .While in antibiotic tests appeard sensitive 80.76%.

Introduction

Simple stomached animal (e.g.pig, chickens, rats and man) lack enzyme that can degrade cellulose or hemicelluloses, and fibrous materials are poorly utilized. Ruminant animal (e.g. cattle, sheep, goats, dare.Etc.) do not synthesis fiber digesting enzyme, but they have formed a symbiotic relation ship with ruminal micro organisms that can. The ruminant provides the micro organisms with habitat for their growth , the rumen and microorganisms supply the animal with fermentation acids , microbial protein and vitamins (1).

Ruminant nutritionists have long been interested in modulating the competition among different microbial populations with the objective of improving the efficiency of energy and protein utilization in the rumen. This has been achieved through the optimization of diet formulation and the utilization of feed additives that modify the environment and enhance or inhibit specific microbial population (2).

Some ruminal bacterial produce lactic acid at a rapid rate , and this acid can cause pronounced declines in ruminate PH, founder , and in sever cases , even death of the animal (3).

The predominant rumen bacterial species occurring in cattle,sheep metabolism in the goat are to be interpreted as similar to the values in cattle and sheep.(4)

Staphylococci display high virulence , other may have acommensal relation ship with their host, staphylococci Forman important component of the bacterial community in the rumen content as well

as on the rumen wall of domestic and wild ruminants.(5,6).

Despite the fact that staphylococci are known also lactic acid producers and belong together with lactobacilli and interococci to the first bacterial group colonizing the rumen of young ruminants (7), they may be important in causing infection under the appropriate prides posing conditions similar to those described by (8) for enterococci.

The aim of this study is isolation and identification of *Staphylococcus aureus* from ruminal content of cows .

Material and methods

Samples collection: A total of 50 samples were randomly collected from cows ruminant content in order to isolate *Staphylococcus aureus.*

Laboratory diagnosis: The specimens were transported to the laboratory directly. In the laboratory the diagnosis was performed by :

A- Culturing :

The specimens were directly inoculated on a plates of Mannitol Salat Agar (MSA) and incubated at 37°c for 24hrs. All colonies from primary cultures were purified by sub culture on (MSA) medium and incubated at 37°c for 24-48 hrs.(9)

B-Microscopic examination :

Asmear was prepared and staind with Gram stain. to see G+ cocci in cluster(10)

Biochemical test:

A. Catalase test : a small amount of pure growth was transferred with a wooden stick from mannitol salt agar to clean slid , then a drop of Catalase reagent was added(hydrogen peroxide.3%.The evaluation of gas bubbles indicates a positive test (11)

B. Coagulase test: This test was done according to (12) by adding 0.1 ml from 18-24 hrs. Culture broth to the 0.1 ml of human plasma without dilution and incubation at 37°c for 4 hrs. the appearance of the clotting indicates a positive result comparable to control.

Biotyping test:

A. Haemolysin production:All bacterial isolates were streaked on blood agar and incubated at 37°c for 24 hrs. Presence of zone of haemolysis around the colonies was considered as appositive tesult (13)

B.Growon Crystal violate medium: This medium was prepared by adding 0.1 ml of crystal violet to brain heart agar to 1 liter D.W.(1:10 000). (14).

C.Pigment production on Milk agar: This media was prepared by dissolve nutrient agar 28gm, in 700 ml D.W. and autoclaving , after that cooling to 55° and supplement with 300 ml of milk and distributed in petri dishes, It was based on method by Christi and Keogh, 1945 and described by (10). It is used to test of pigment production.

Biotyping:

The isolates of *S.aureus* were cultured on the Milk Agar and incubated at 37°C for 24hrs. The appearance of yellow pigment was indicated as appositive results. Depending on the results to Coagulase production , culturing of isolates on the crystal violate medium different biotypes of four classified depending on the color of the colonies on this medium,biotype A appeared in acrystal violate color, biotype B appeared white on, biotype C appear yellow while biotype D did not grow on this medium.(14)

Antibiiotic susceptibility Test:

This test was done according to method of (15). pure colony transferred to clean tube contain 4 ml Brain Heart infusion broth and incubated at 37°c. After moisten the swab in the broth culture and the swab was passed on the surface of the Muller-Hinton agar from center to the border , the

plate was left for 15 min. to be dry , the discs of antibiotics were fixed on the plate by using sterile forceps , after incubation at 37°c for 24 hrs.The inhibition zone was measured and compared to special standard table containing.

Results:

The results showed that 26/50 (52%) of Cows rumen content gave positive result (able to grow) on mannital salt agar (MSA) after 24 hrs. the suspected colonies of *S. aureus* were , round, smooth, raised, glistening, gray to deep golden yellow in color, , the smear of suspected colonies showed Grape like cluster G+ cocci. The result of biochemical and growth tests revealed that all detected isolates were *S.aureus* (100%).

Table showed that 69.23% of isolates were B- hemolytic and 61.5% isolates were C biotype, while the pigment production on milk agar was observed in 76.92% of isolates.

Table (1): Number & percentage of *S.aureus* biotypes

BIOTYPING TEST	NO. OF BIOTYPE	PERCENTAGE	TOTAL
Beta- Heamolysin	18	69.23%	26
Alfa- Heamolysin	8	30.76%	
Biotype C	16	61.5%	26
Biotype A	10	38.4%	
Pigment	20	76.92%	26

Table (2) display the susptibility of *S.aureus* isolates against (10) antibiotics. Mast isolates showed higher susptibility rate to Ciproflaxcin 80.76% and all tstes isolated showed higher rate of antibiotic resistance 96.15% against the Metronidazol.

Table (2) : The antibiotic sensitivity test results

ANTIBIOTIC	SENSITIVE ISOLATS	RESISTANCE ISOLATS
Ttobramycin	18(69.23%)	5(19.23%)
Chloramphenicol	20(76.92)	4(15.38%)
Enrofloxacin	9(34.61%)	12(46.15%)
Vancomycin	10(38.46%)	14(53.84%)
Gentamicin	20(76.92%)	0(0%)
Erythromycin	5(19.23%)	15(57.69%)
Ciprofloxacin	21(80.76%)	3(11.53%)
Penicilin	4(15.38%)	21(80.76%)
Metrondazol	0(0%)	26(100%)
Kanamycin	17(65.38%)	5(19.23%)

Discussion:

The rumen is inhabited by bacteria , protozoa and fungi, but bacteria play a dominant role in all facts of ruminal fermentation (16) Gram- Positive bacteria produce more ammonia, hydrogen and lactate then Gram- Negative species, and compounds that inhibit Gram-Positive ruminal bacteria have increased feed efficiency (17)

The present study aimed to detect or investigate the presence of *S.aureus* in 50 samples of rumen. *S.aureus* was diagnosed in an over all rate (52%).

The presence of this bacteria in Basrah was supported by study conducted previously in Basra by Basim,(2009) who reported this microorganism was isolated from bovine in percentage 63.36% .

According to production of Pigmen ,Heamolys ,Coagulase of bovine plasma and growth on crystal violate, *S.aureus* was classified in present study in to biotype C 61.5% and biotype A 38.4% .This result was in line with the findingof Hanon(18) who reported the biotype which is specific to bovine isolates with percentage 83.33% and biotype A which specific to human in 16.66% of isolates .

Also these result was agreement with (19) who, mentioned that the majority of *S.aureus* isolates from ewe milk and cheese were found to be bovine biotype 62% Bendahou *et,al*.(20) showed that the four biotype A,B,C, unspecific, *S.aureus* isolates of milk and milk products appeared bovine origin , biotype C with the percentage 45% and more dominant then other biotypes.

The present results showed the ability of *S.aureus* isolates to produce Beta-haemolysis 69.23% , these result was in line with finding of (21) , who reported that the percentage of B- haemolysin production were 84.9% in bovine isolates . while this results were in compatible with the results of (22),who found the percentage of B-haemolycin in ovine 82.25% , cows 72.7% and equine 72% with total percentage 78.2% but the study disagreed with (23) found isolates from clinical and sub clinical mastitis in cows produce Alfa – haemolysis in 89.79%.

This study revealed different percentage of susceptibility to different antibiotic and showed that 80.76%, 76.92%, 76.92%, 69.23%, 65.38% of *S.aureus* isolates were sensitive to Ciprofloaxicin, Choralphenical, Gentamycin, Tobramycin and Kanamycin respecitivily. This result was agreement with (23) who reported that *S.aureus* isolated from bovine were highly sensitive to Gentamycin 70.37%, Streptomycin 64.63%, Erythromycin 50%,chloramphenical 43.9% and had high resistance to Penicillin 96.13% .In the study of (24) recorded that *S.aureus* isolated from mastitis cows appeared to be highly sensitive for Ciprofloxacin ,Chloramphenical and Gentamycin.

High resistance appeared to Penicilin and Cloxacillin. But the study of (25) recorded high sensitivity of *S.aureus* isolates from Mastitis to Ciprofloxacin 100%, Gentamycin 76.8% and Chloramphenical 41.04%. The high percentage of rssistance appeared to Penicilin 98.27%, and all strain showed resistance to Vancomycin Bendahou *et,al*. (20) mentioned that *S.aureus* isolated from raw milk and milk product appeared sensitive to Vancomycin 100% Chloramphenical 90%, Erythromycin 80%, and high resistance to pencilin (22). Al-Rahman (26) found the high susceptibility to Gentamycin 100%, Tobramycin 90%, Chloramphenical 88% Erythromycin76% and less sensitive for Penicilin 26% in isolates from Camel.

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عزل وتشخيص المكورات العنقودية الذهبية من محتويات كرش الأبقار

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الخلاصة

أجريت هذه الدراسة لعزل المكورات العنقودية الذهبية وتشخيصها من محتويات كرش الأبقار من خلال اخذ عينات وزرعها على أكار المانيتول الملحي وسط (MAS) وقد كانت نسبة العزل ٥٢% أجريت بعض الاختبارات الكيموحيوية التي أكدت كون العزلات مكورات عنقودية ذهبية وكانت نسبتها ١٠٠% ، أجريت الاختبارات biotyping حيث كانت من نوع C 61,5% أما نوع A 38,4% وأعطت نسبة 76% للاختبار الصبغات على وسط الحليب. أما بالنسبة للاختبارات المضادات الحياتية فظهرت أعلى نسبة تثبيط لمضاد الحيوي Ciproflaxcin النسبة 80,76% .