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COMPARATIVE STUDIES ON DIFFERENT MEDIA ISOLATION OF PATHOGENIC STAPHYLOCOCCI FROM RETAIL MILK

(With One Table)

By

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المقارنه بين الاوساط الغذائيه المختلفه لعزل الميكروب المكور العنقودى الضار من الالبـــان

هشـــام نـــــدا

يدور البحث حول جمع ٧٥ عينة لبن من موزعى وبائعى الالبان فى منطقتى الدقى والمهندسين - والقيام بزرعها على الأوساط الغذائيه الآتيه :

١ - الآجار الدموى .

٢ - شابمان مانيتول آجار .

٣ - بيرد باركر مستحدث .

وتم المقارنه بين هذه الأوساط الغذائيه فى عزل الميكروب المكور العنقودى الذهبى الضار المذيب للدم وتمت دراسة درجة التجلط .

وقد وصلت النسبه المئويه فى عزل الميكروب من عينات اللبن موضوع الدراسه على التوالى ١٠٪ ، ٣٢٪ ، ٨٠٪ ، على الاوساط الغذائيه المشار عاليه - كما وضح البحث دور مكونات الوسط الغذائى شابمان مانيتول آجار والبيرد باركر المستحدث .

SUMMARY

75 milk samples were collected from various retailers in Dokki and El-Mohandesin. The samples were inoculated onto *Blood Agar Medium, Chapman-Mannitol Medium and Modified Baird-Parker Medium* to evaluate the usefulness of these media for isolation of pathogenic staphylococci from retail milk. This clearified that the incidence of positive milk samples on Blood agar were 20% and on chapman-Mannitol Medium 32%. Meanwhile the *Modified Baird-Parker medium* within its ingred-ients content gave a high number of positive milk samples contaminated with *Staphylococcus aureus* (80%).

Keywards: Different media, isolation pathogenic Staphylococci, retail milk

INTRODUCTION

Staphylococci are one of the most widely prevalent microorganisms, commonly found in milk.

Staphylococci are able to elaborate the enterotoxin in milk as foodstuff. Special bacteriological methods are necessary to distinguish potenially pathogenic staphlococci from harmless ones found in normal udders and on the skin and mucous membranes of man animals. Milk provide excellent media for staphulococcal proliferation. The principal health hazard from infection of milk with staphylocci lies in the production by some strains of an enterotoxin that can cause gastroenteritis in man consuming such milk.

Staphylococci are ubiquitous organisma and at least 50% of individuals carry the organisma from time to time in their nasal passages, throats through cough or sneeze, and on hand's handlers or retailers; *SUSAN, et al. (1993).*

The coagulase positive staphylococci are responsible for cases of intoxication associated with consumption of milk *(MOSDOL, 1978).*

This work aims to study the incidence of *Staphylococcus aureus* in milk .

Some workers are directly inoculate the sample onto blood agar, others use the manitol salt agar or preenrichment in chapman broth and Baird-Parker media in order to inhibit the growth of organisms other than staphylococci, this work also

refers to the comparison of the senstivity and specificity of different media used for isolation of staphylococci from milk *(RUFFO, 1968).*

MATERIAL AND METHODS

75 samples of milk collected from differnet retailers in Dokki and El-OMohandesin, Giza, were used for the experiment.

Bacteriological examination:

The samples were incubated at 37 °C for 1 hour, then 2ml of each sample were directly streaked on *Blood agar.* The plates were incubated at 37 °C for 48 hours then examined for haemolysis, *FOX, et al. (1992).*

The same samples were inoculated into chapman broth incubated at 37 °C for 24 hours, then a subculture was made on *Chapman medium (mannitol salt agar).* Growth and change of the colour of the medium into yellowish colour indicates the presence of staphylococcus aureus suspected colonies as *Staphylococcus aureus* ferment manoitol *(RUFFO, 1968).*

The same samples were inoculated onto Baird-Parker medium then incubated at 37 °C for 24 hours.

The growth of staphylococci indicated by the presence of black precipitate and blackening of the medium, *(OXOID MANUAL, THIRD EDITION 1973)* due to the presence of potasium telllurite.

Preparation of different media used in this study as well as identification of the developed cultures were preformed according to the system described by *CRUICKSHANK (1982).*

RESULTS

The results of bacteriological examination of 75 raw milk samples collected from retailers are shown in Table 1. The table clearified the samples confirmed as staphylococcus aureus "coagulase positive and haemolytic" as the followings:

On Blood Agar 15 (20%) on *Chapman Mannitol Agar* 24 (32%) on *Modififed Baird-Parker* 60 (80%). This showed that in naturally contaminated milk cultured on *Blood Agar Media* gave the lowest percentage of isolation, also the microscopical examination of haemolytic colonies were due to growth of staphylococci but due to coccobacilli and anbthracoids.

The same samples of raw milk cultured of *Chapman Mannitol media* contribute at the earsier growth of staphylococci (golden yellow culture) and inhibit other microorganisms) due to the action of the action of the effective components of this media

which gave higher percentage in isolation of *staphylococcus aureus* (coagulase positive) than blood agar media as shown on the table.

Also as shown in the table, Modified Baird-Parker Media used in this work showed high percentage of isolation of *staphylococcus aureus* (coagulase positive) from the same examined raw milk samples in comparison to both blood agar and Chapman-mannitol media.

The action of the componentss of Baird-Parker mediunm can be summarized as follows:

- Lithium chloride inhibit the growth of gram negative flora.
- Potassium tellurite inhibit the gram positive flora.
- Glycine, sodium pyruvate affects the anaerobes.
- Egg yolk act as emulsion stimulant and physical process of stabilization. Egg yolk gave a suitable opaque colonies whitish zoen of clearing.

Therefore the use of modified Baird-Parker medium in this work permits the detection of very small numbers of *Staphylococcus aureus* which appears as blackish precipitate.

Besides this modified Baird-Parker medium save time for isolation and identification of staphylococci and gave more quicker and accurate results than any other methods.

The results of examination of *staphylococcus aureus* naturally contaminated milk samples can be summarized as on the following table.

DISCUSSION

The results of bacteriological examination for staphylococci as shown on the table of this work confirmed as coagulase positive strains of staphylococci and cultured on chapman-mannitol salt agar medium (32%) were agreement to *RUFFO, (1968)* who found (35.66%) staphylococii on chapman-mannite medium.

Meanwhile cultures of the same milk samples on the blood agar medium showed 15 (20%) haemolytic *staphylococcus aureus* out of 75 milk samples.

So isolation of such haemolytic *staphyloccus aureus* as shown in this work reflects the role of pathogenicity of the organism to the consumers. These findings is in agreament to that work reported by *MOSDOL (1978).*

In regard to Chapman-Mannitol agar media, pathogenic staphylococci (coagulase-positive) are able to grow on high salt mannitol media to form organe colonies which gave positive reactions for acid production from mannitol.

In relation to culturing the same milk samples onto Baird-Parker medium to be used for the determination of staphy-lococcus type, 80% of the samples gave positive cultures. This indicate the importance of using such medium for isolation and identification of *staphylococcus aureus* from raw milk due to the development of characteristic black precepitate *(ANTILA, et al. 1979)*. These findings are inagreement with *MAHMOUD, et al. (1980)* as well as *VIANNI, et al. (1989)* who prefer the Baird-Parker media in identificantion of staphylococci aureus from raw milk.

VIANNI (1989) showed in his work that the incidence of *staphylococcus aureus* isolates was 50% on Baird-Paker medium, meanwhile in this work as shown on the table, the incidence reached 80%. Enrichement with egg-yolk tellurite emulsion seems to be of its physical importance in stabilisation of the medium. Baird-Parker medium and egg yolk gave a suitable opaque against whitish zones of clearing can be easily detected as reported by *VIANNI, et al. (1989), FOX, et al. (1992), SUSAN, et al. (1993)*.

Woreover the effect of the components of Baird-Parker media, as lithium chloride which inhibit gram negative flora, potassium tellurite acts on gram positive flora while, the presence of glycine, sod. pyruvate affects the anaerobes, *ANTILA, et al. (1979)*. Such type of supplement facilitate the isolation of pure cultures of staph. aureus from raw milk samples.

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Table 1: Isolation of staphylococci from examined milk samples.

Blood Agar Medium		Chapman-Mannitol Medium		Pa i rd-Parker Medium	
No. of examined milk samples	75	No. of examined milk samples	75	No. of examined milk samples	75
Haemolytic	30	Change of media	30	Black ppt	75
+ve Staph. by M.E	45	+ve Staph. aureus(golden yellow colony)	24	+ve samples for Staph. M.E	75
Haemolytic Staph.	15	%	32	+ve samples for Staph. aureus.	60
Non-haemolytic Staph.	30			%	80
+ve Staph. aureus	15				
%	20				

M.E = Microscopical examination