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دراسة بكتريولوجيه عن التهاب الضرع الخفي في أبقار بمزرعة الهرم مع التركيز على حساسية الميكروبات للمضادات الحيوية

صبرى توفيق ، سمير ناشد ، فوزى جودة * ، سميره الجبالي
و عادل فريد ، سعد ندا

أمكن عزل ١٢٢ عترة من عينات لبن عشوائية سليمة ظاهريا من ١٠٣٤ بقرة فريزيان حلوبة بنسبة ٨ر١١٪ .

الميكروبات المعزولة كانت كالاتي :-

- الميكروب العنقودى المرضي (٦٦ر٦٠٪) .
- الميكروب السبحي (١٤ر٣١٪) وتشمل على الميكروب السبحي اجلاكتيا (٣ر١٦٪) ، الميكروب السبحي د س اجلاكتيا (٤ر١١٪) ، الميكروب السبحي يوبرز (٢ر٣٪) ، بالاضافة الى الميكروب القولوني والكورينيي بيوجين بنسبة ٩ر٤٪ ، ٢ر٣٪ بينما الاصابة المزدوجة ٢ر١٥٪ .

الميكروبات المعزولة كانت حساسة للمضادات الحيوية في ترتيب تنازلي للكاناميسين ، ايروتوميسين وبولي مكسين ، ستربتوميسين ، بنسلين .

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PRELIMINARY STUDIES ON BACTERIA RECOVERED FROM MILK OF APPARENTLY HEALTHY UDDERS IN DAIRY CATTLE AT EL HARAM AREA WITH SPECIAL REFERENCE TO ANTIBIOTIC SENSITIVITY

(With 5 Tables)

By
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SUMMARY

Milk samples were randomly collected from apparently healthy udders of 1034 milking Friesian cattle for bacteriological examination. 122 atrains were isolated with a recovery rate of 11.8% of the samples. Microorganisms secured were coagulae-positive Staphylococci (60.66%), Streptococci (31.14%) including Str. agalactiae (16.3%), Str. dysgalactiae (11.4%) and Str. uberis (3.2%). In addition, E. coli and C. pyogenes were recovered with an incidence of 4.9% and 3.2% respectively while the recovery rate of double infection was 51.2%.

The secured organisms were sensitive to the following sntibiotics in a descending order: Kanamycin, Erythromycin, Polymyxin, Streptomycin and Penicillin.

INTRODUCTION

Subclinical mastitis acts as an obscure source of infection. It is necessary to detect the disease as early as possible, because of its public health importance, since streptococci and staphylococci which are pathogenic for human being, may be excreted in milk and give rise to scarlet fever, septic sore throat, pyogenic infection and food poisoning to those consuming raw milk (St. GEORGE et al. 1962; WILSON et al. 1966).

Mastitis has been considered as a major problem for the dairy industry as expressed in early meetings held on animal diseases in WARSOW (1948). The infected quarters look apparently normal and could act as an invisible source of infection among the dairy cattle. O'DONOVAN et al. (1960) estimated that subclinical mastitis was associated with a drop of 10% milk yield, 11% in non-fatty solid and 12% of butter fat in an infected herd.

A survey done by KHALIL et al. (1968) on subclinical mastitis revealed 50 (14.9%) positive cases out of 336 milk samples, examined form apparently normal udders. of these, 5 samples (10%) showed double infection (1 coagulase-positive Staph., 3 Str. dysgalactiae, 1 intermediate coliform and 1 Aerobacter aerogenes). The remaining 45 samples (90%) were single infection (1 coagulase-positive Staph. 33 Streptococci including 18 Str. agalactiae, 11 Str. dysgalactiae, 4 Str. uberis and 11 coliforms).

Microorganisms recovered from mastitis were sensitive to certain antibiotics in a variable degree (RAE and NAIDU, 1969).

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The aim of this work was to detect the incidence of microorganisms recovered from milk samples collected from apparently healthy udders, in addition to the antibiotic sensitivity of secured organisms in vitro.

MATERIAL and METHODS

1034 individual milk samples were randomly collected from apparently normal udders of Friesian cattle at different breeding stations at El-Haram, which were free from Tuberoulosis and Brucella. The teats and udders were washed with sterile water, then treated with doches of potassium permenganate 1/1000 dilution. The first few streaks of milk were discarded. Ten ml. of milk sample were collected aseptically in sterile test tube for bacteriological examination. Samples were centrifuged for 15 minutes at 3000 r.p.m. Loopfuls of the sediment were streaked on 10% sheep blood agar, MacConkey's agar (Difco) and Edwards medium and incubated at 37°C for 48 Hrs.

Pure colonies were subcltured on blood agar, nutrient agar and identified according to BREED et al. (1957), BAILY and SCOTT (1962), and EDWARDS and EWING (1969).

Sensitivity tests were applied on the secured organisms, using five antibiotics: Kanamycin sulphate (30 mg), Erythromycin (15 ug), Penicillin (10 i.u), Polymyxin (300 u) and Streptomycin (10 mg). An overnight culture was prepared and diluted with sterile saline to a density equivalent to barium sulphate standard (BLAIR et al. 1970). The entire surface of nutrient agar plate (Difco) was streaked evenly with sterile cotton swab, soaked with the standardized 24 h broth culture, left to dry at room temperature for 30 minutes. Sensitivity disks (Difco) were aseptically applied with sterile flammed forceps, 3 cm. apart from each other, then incubasted at 37°C for 24 hr. Zones of inhibitions around the disks were measured in mm. with a ruler.

RESULTS

Out of 1034 milk samples, 122 strains were recovered. The incidence of infection, number and species of microorganisms were recorded in Table 1 and 2. Staphylococci was present in a higher incidence (60.66%) than streptococci (31.14%). The ratio of single and double infection was recorded in Table 3. The interpretation and the methods of antibiotic susceptibility were carried out after BAUER et al. (1966) in Table 4.

The sensitivity of microorganisms to the antibiotics used was illustrated in Table 5.

DISCUSSION

It is known that there are predisposing factors which lower the resistance of the udder for bacterial invasion, the most important of which are the general health of the animal, the milking machine, the unhygienic condition or the bad milking habits. In the present survey, it sems that the incidence of mastitis as expressed in terms of recovery rate, reached 7.9%.

In an earlier survey, KHALIL et al. (1968) recorded an incidence of 14.9% of subclinical mastitis. ABDEL KARIM and EL-ASHMAWY (1979) and AHMED (1981) recorded that the incidence of subclinical mastitis was 56.43% and 76.79% respectively. It is generally recognized that Str. agalactiae and Staph. aureus are among the prevalent microbes causing mastitis in dairy cattle (SCHALM and LASMANIS, 1957). Different types of Streptococci recovered in the present work, are of great importance since they cause mastitis as shown earlier by LITTLE (1937), FOLEY et al. (1948), HUGHES (1953), HALE et al. (1956) and POBERTS et al. (1963). Str. agalactiae

was revealed in the present work in a lower incidence (16.3%), while ROBERTS et al. (1963) and KHALIL et al. (1968) isolated Str. agalactiae in an incidence of 46% and 42% respectively.

Among the most important factors that explain the high incidence of Streptococcus mastitis in the udder, is the infection from nasopharengeal passages of the milkers or other human carriers. The presence of these types of bacteria in the milk is likely to be followed by outbreaks of sore throat or scarlet fever in persons consuming such milk in the raw condition.

Although C. pyogenes plays a role in summer mastitis, yet it is rather frequent during the 2nd half of the year (WILSONS and MILES, 1963). It is more frequent in dry cows than in cows in milk and develops shortly before calving. However, it was isolated in the present investigation in a recovery rate of 3.2%.

With regard to the recovery rate of secured organisms in single and double infection in apparently healthy udders the results were variable.

FUJIKURA and SHIBATA (1965) noticed that out of 232 cases of positive California mastitis tests in cows, the incidence of Staph. aureus was 72.4% in single infection while in double infection with Staph. aureus and Streptococci was 12.5%. In Streptococcus infection together with other bacteria, the incidence was 6.4%. The same authors reported, that out of 372 negative California mastitis test, the incidence of Staph. aureus in single infection was 48.3%, while it reached 12.9% in double infection with Streptococci and Staphylococci. In Streptococcus infection together with other bacteria, the incidence was 7.2%. It is worthmentioning to point out from the present results that the recovery rate of coagulase positive Staph. in single infection was 29.5%, while it reached 30.9% in double infection with Streptococci and Staphylococci (Table 2 & 3).

Referring to coliform infection in subclinical mastitis cases SCHALM and WOODS (1952) isolated E. coli from apparently normal udders. The present findings coincide with those earlier reports since the recovery rate was 3.2% and 21.6% in single and double infection respectively. The detection of E. coli in apparently normal milk has its public health importance, since certain strains are responsible for infantile gastro-enteritis.

It would appear that, th coli-infection of the udder is due to improper cleanliness or hygiene of the barn where faecal droplets are allowed to settle on the udder.

Looking at the sensitivity of microorganisms causing mastitis to certain antibiotics, FARRAG and OOF (1967) used eight antibiotics to 26 strains of C. pyogenes (I), five of C. ovis (II), 15 Staph. aureus (III), 14 Str. agalactiae (IV), 12 E. coli (V) and nine Pseud. aeruginosa (VI) isolated from cows and goats with mastitis in Egypt. Tetracycline and chloramphenicol gave the best results in inhibiting most organisms apart from (VI). Penicillin, Erythromycin and novobiocin had no inhibitory affect on V and VI. Streptomycin inhibited V and VI, while its effect on other microorganisms was variable. Group I, III and V showed moderate resistance to all antibiotics in use.

In is evident that Erythromycin (Table 5) had no inhibitory effect on Str. dysgalactiae, Str. uberis and E. coli, while Str. agalactiae and C. pyogenes were moderately sensitive, a finding which agreed with FARRAG and OOF (1967).

Isolates recovered in the present work were resistant to penicillin except coagulase-positive Staph and Str. agalactiae which were slightly sensitive. On the other hand, Streptomycin was effective on E. coli, while the majority of strains were resistant.

Kanamycin was effective to a certain degree on all organisms, recovered in this study, which coinside with FARRAG & OOF (1967). On the other hand, Polymyxin and Erythromycin were effective on all strains in a variable degree except Str. uberis.

It is concluded that Kanamycin, can be considered as the drug of choice, however, its value in the field and its effect in vivo need further investigation.

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Table (1)
The incidence of infection in Friesian cattle
in El-Haram area.

No. of samples collected	No. of samples infected	%	No. of strains insolated	%
1034	82	7.9	122	11.8

Table (2)
Microorganisms recovered from apparently healthy udders

Microorganism	No. of strains	Percentage	
Str. agalactiae	20	16.393 %	
Str. dysgalactiae	14	11.475 %	31.146
Str. uberis	4	3.278 %	
Coagulase-positive Staph.	74	60.660 %	
E. coli	6	4.920 %	
C. pyogenes	4	3.278 %	

Table (3)
Microorganisms recovered from udders with single and double infections

No. of samples infected	Single or double infection	Str. agalactiae	Str. dysgalactiae	Str. uberis	coagulase-positive Staph.	E.coli	C. pyogenes
42	Single				36 (29.5%)		
	Single					4 (3.2%)	
	Single						2 (1.6%)
40	Double	20 (16.3%)			20 (16.3%)		
	Double		14 (11.4%)		14 (11.4%)		
	Double			4 (3.2%)	4 (3.2%)		
	Double					2 (1.6%)	2 (1.6%)

Table (4)
Interpretation of the antibiotic succeptibility after Bauer et al. (1966)

Antibiotic	Dise potency	Inhibition Zone diameter to nearest mm		
		Resistant	moderate	Sensitive
Kanamycin	30 mg	13 or less	14-17	18 or more
Penicillin	10 u	11 or less	12-23	23 or more
Polymyxin B	300 ug	8 or less	9-11	12 or more
Streptomycin	10 ug	11 or less	12-14	15 or more
Erythromycin	15 ug	13 or less	14-17	18 or more

Table (5)
The sensitivity of microorganisms to antibiotics

Antibiotics	Str. agalac-tiae	Str. dysgalac-tiae	Str. uberis	congulase-positive Staph.	E.coli	C.pyogenes
Kanamycin	++	++	+	++	+++	++
Penicillin	++	+	+	++	+	+
Polymyxin	++	++	+	+	+++	+
Streptomycin	++	+	+	+	+++	+
Erythromycin	++	+	+	+++	+	++

Sensitive = +++
Moderate sensitive = ++
Resistant = +