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BACTERIOLOGICAL EVALUATION OF VAGINAL DISCHARGES IN COWS WITH ENDOMETRITIS AND CLINICALLY HEALTHY HEIFERS IN ASSIUT GOVERNORATE

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ABSTRACT :

The study was carried out for the isolation and identification of vaginal bacterial flora of 36 healthy and diseased cows in a freisian dairy farm in Assiut Governorate. The animals were divided into 4 categories: the first included 6 repeat breeder cows with endometritis after last birth, the second included 13 pregnant heifers, the third included 12 heifers at the age of mating and the last one included 5 primiparus cows. 50 bacterial strains were identified from the vaginal discharges. These isolates were: 15 (30%) *E.coli* , 11(22%) *Staphylococcus epidermidis*, 2 (4%) *Staphylococcus saprophyticus*, 1 (2%) *Streptococcus pneumoniae*, 10 (20%) *Corynebacterium sp.* 8 (16%) anthracoid bacilli, 1 (2%) from each of *Enterobacter aerogenes*, *Klebsiella oxytoca* and *Citrobacter diversus*. Antibiotic sensitivity tests were carried out against the isolated microorganisms using ten antibiotics. It was found that cibrofloxacin was highly effective against most strains (92%) followed by Gentamycin (90%) and amoxycillin (74%) while cloxacillin and duricef were not effective (0%).

INTRODUCTION :

The importance of microorganisms as the etiologic agents of infertility is well recognized (Robert, 1971). The degree of resistance of cattle to non-specific genital infection is related to the endocrine state prevailing at the time of infection. Thus, at oestrus and at parturition, the resistance is highest but lowest during pregnancy and dioestrus, that is during the luteal phase and therefore uterine infection is most likely to become established. Normally there is a bacterial flora of the vagina of healthy cows but the bacterial population is kept within bounds by the defense mechanism. The causal organisms of endometritis may reach the uterus from the vagina and cervix or from the blood stream. Infection from the vagina is likely at service and at parturition by organisms of the normal flora peculiar to the genital tract of the cow and the penis of the bull. (Arthur, 1975).

Bacterial organisms that cause endometritis in cattle suffering from repeat breeding occupy the highest percentage among the other causes.

(Cupps, 1973). Luft (1976) studied the fertility status of a dairy herd of cows and found that 67% were repeat breeders as a result of endometritis.

Otto (1986) mentioned that husbandry and sanitation practices commonly employed in dairy cows at parturition expose the uterus to a broad range of bacterial contamination and provide an increased opportunity of cows to develop vaginal discharge, the uterine infection often resolve spontaneously.

Takale, *et al.* (1994) concluded that the normal bacterial flora of the genital tract may become pathogenic under favourable conditions.

Therefore the aim of the present study was first to isolate and identify the bacteria which may be responsible of endometritis in repeat breeder cows and subsequently to determine the predominant groups of bacteria capable of colonizing the vagina of apparently healthy heifers. The antibiogram for the isolated bacterial flora was also studied.

MATERIAL AND METHODS :

MATERIAL:

1- Animals:

36 cows were choosen from a friesian farm in Assiut Governorate. The animals were classified into 4 groups:

***1st group included 6 cows suffering from endometritis resulting in repeat breeders.**

***2nd group included 13 pregnant heifers, their gestation period ranged from 3-8 months.**

***3rd group included 12 non pregnant heifers at age of mating (18-24 months)**

*** 4th group included 5 primiparus cows.**

2- samples:

Vaginal cotton swabs were taken under complete aseptic conditions. In case of parturition the sample was taken within 4-10 days after parturition.

METHODS:

1- sampling:

Vaginal swabs were taken (after gynecological examination per rectum) under complete aseptic conditions and then sent to the laboratory with no delay to avoid dryness of samples.

2- Bacteriological examination:

a- Isolation and identification:

Swabs were inoculated into nutrient broth and incubated over night at 37° C. Loopfuls were subcultured onto 5% sheep blood agar and MacConkey's agar and the plates incubated for 24 hr. in. The growing colonies were described morphologically and microscopically. For further identification biochemical reactions were done after colonial purification according to Ellen *et al.* (1994) and Quinn *et al.* (1994).

b- Serotyping of *E.coli* strains:

Isolated strains which identified biochemically as *E.coli* were subjected to serotyping using 9 available antisera produced by Difico Laboratories following the instructions of the producers. These antisera were O:26 ab, O:55, O:86a, O:111, O:119, O:124, O:125 ac, O:126, O:128,

3- Antibiogram:

It was carried out by the standard diffusion technique for the isolated strains against 10 different antibiotics [Ampicillin (10 µg), Amoxycillin (10 µg), Cefteriaxone (30 µg), Cloxacillin (5 µg), Ciprofloxacillin (5 µg), Duricef (30 µg), Erythromycin (15 µg), Gen-

tamycin (10μg), Penicillin (10 units), Streptomycin (10 μg)]. Categorizing the tested strains, as sensitive or resistant, was based on the measurement of the diameter of inhibition zone obtained according to Bauer-Kirby scale (Atlas, 1995).

RESULTS :

The obtained results are shown in tables 1-4 and Figures 1-3.

Table (1): Bacteriological examination of the animal groups.

Group	No. of examined animals	Presence of bacteria				No. of isolated strains	No. of animals with			
		Present		Absent			single isolate		2 isolates	
		No.	%	No.	%		No.	%	No.	%
1 -Cows with endometritis	6	6	100	0	0	11	1	16.66	5	83.33
2-Pregnant heifers	13	12	92.30	1	7.69	21	3	25.00	9	75.00
3-Non pregnant heifers	12	12	100	0	0	12	12	100.0	0	0.00
4-Cows with recent parturation	5	3	60.00	2	40.0	6	0	0	3	100.0
Total	36	33	91.66	3	8.33	50	16	48.48	17	51.51

Table (2): Different isolated microorganisms from the 4 groups of animals.

Group	Total isolates	Staph. sp.		Strept. pneumoniae		Coryne sp.		Anthracoid bacilli		Enterobacteriaceae	
		No.	%	No.	%	No.	%	No.	%	No.	%
1	11	3	27.27	1	9.09	7	63.63	0	0.00	0	0.0
2	21	4	19.04	0	0.0	3	14.28	0	0.00	14	66.66
3	12	3	25.00	0	0.0	0	0	8	66.66	1	8.33
4	6	3	50.00	0	0.0	0	0	0	0.0	3	50.0
Total	50	13	26	1	2.0	10	20	8	16.0	18	36.0

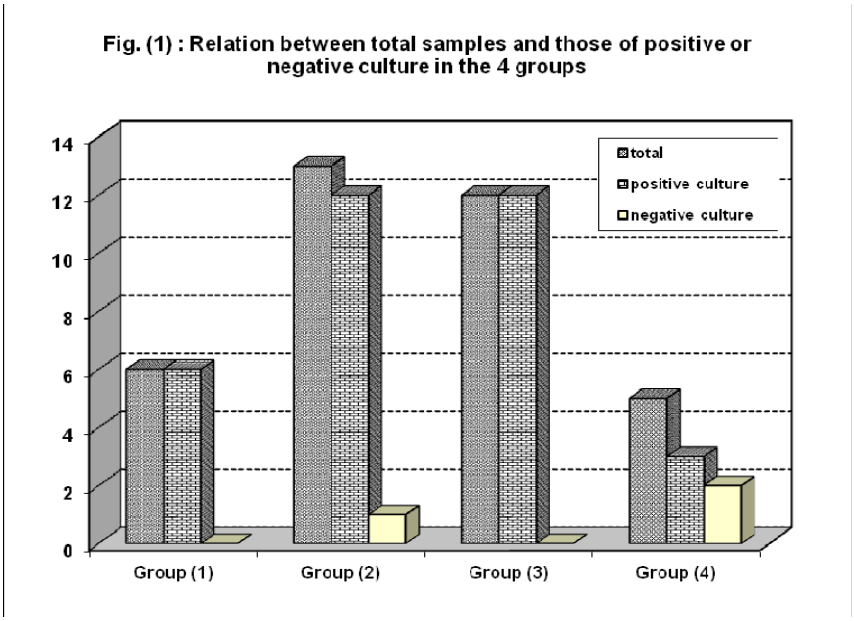
Table (3): List of microorganisms isolated from 36 cows.

Isolated microorganisms	No.	%
Staphylococcus epidermidis	11	22
Staphylococcus saprophyticus	2	4
Streptococcus pneumoniae	1	2
Corynebacterium sp.	10	20
Anthracoid bacilli	8	16
Untyped *E. coli*	15	30
Enterobacter aerogenes	1	2
Klebseila oxytoca	1	2
Citobacter diversus	1	2
Total	50	100

Table (4): Susceptibility of the isolated strains to the different antibiotics.

Species	No.	Ampicillin	Amoxycillin	Ceftrixone	Cloxacillin	Cipro floxacin	Duricef	Erythromycin	Gentamycin	Penicillin	Streptomycin
E.coli	15	n 0 % 0	11 73.33	4 26.66	0 0	12 80.0	0 0	8 53.33	12 80.0	7 46.66	4 26.66
S. epidermidis	11	n 4 % 36.36	7 36.36	3 27.27	0 0	11 100	0 0	0 0	11 100	0 0	3 27.27
S.saprophyticus	2	n 0 % 0	0 0	1 50	0 0	2 100	0 0	0 0	1 50	0 0	0 0
Strept. sp	1	n 0 % 0	0 0	0 0	0 0	1	0 0	0 0	0 0	0 0	0 0
Anthracoid	8	n 0 % 0	8 100	4 50	0 0	8 100	0 0	3 37.5	8 100	0 0	8 100
Coryne. spp.	10	n 5 % 50	9 90	6 60	0 0	10 100	0 0	0 0	10 100	0 0	0 0
Enterobacter sp.	1	n 0 % 0	1	0	0	1 100	0 0	0	1	0	1
Klebsiella sp.	1	n 0 %	0	0	0	1	0 0	0	1	0	0
Citrobacter sp.	1	n 1 %	1	0	0	0	0 0	0	1	0	1
Total	50	n 10 % 20	37 74	18 36	0 0	46 92	0 0	11 22	45 90	7 17	17 34

N.B.:
n = number of susceptible strains. **% = percentage of susceptible strains.**



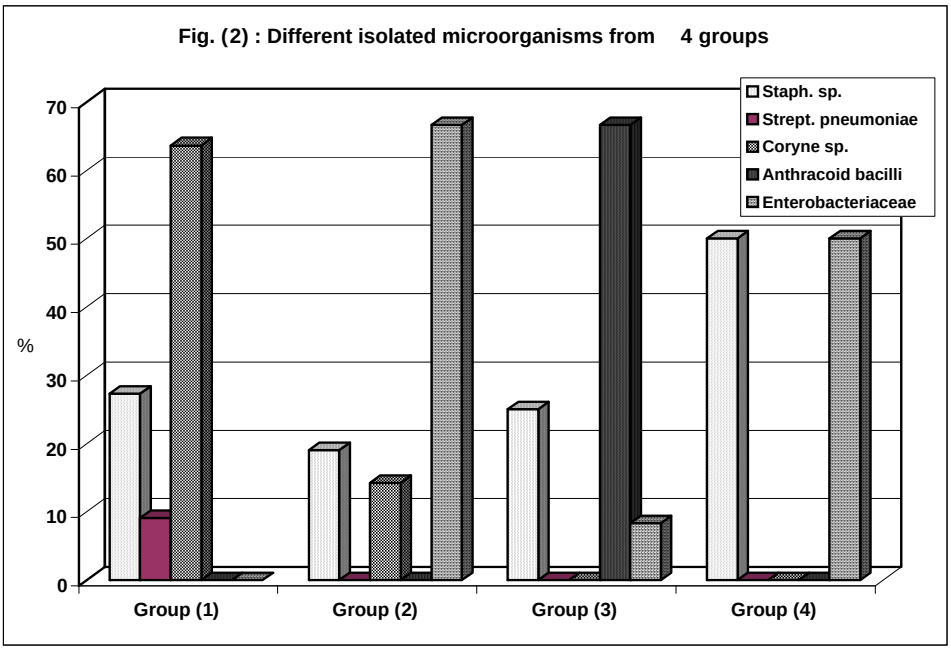


Fig. (2) : Different isolated microorganisms from 4 groups
Staph. sp.
Strept. pneumoniae
Coryne sp.
Anthracoid bacilli
Enterobacteriaceae
%
0
10
20
30
40
50
60
70
Group (1)
Group (2)
Group (3)
Group (4)

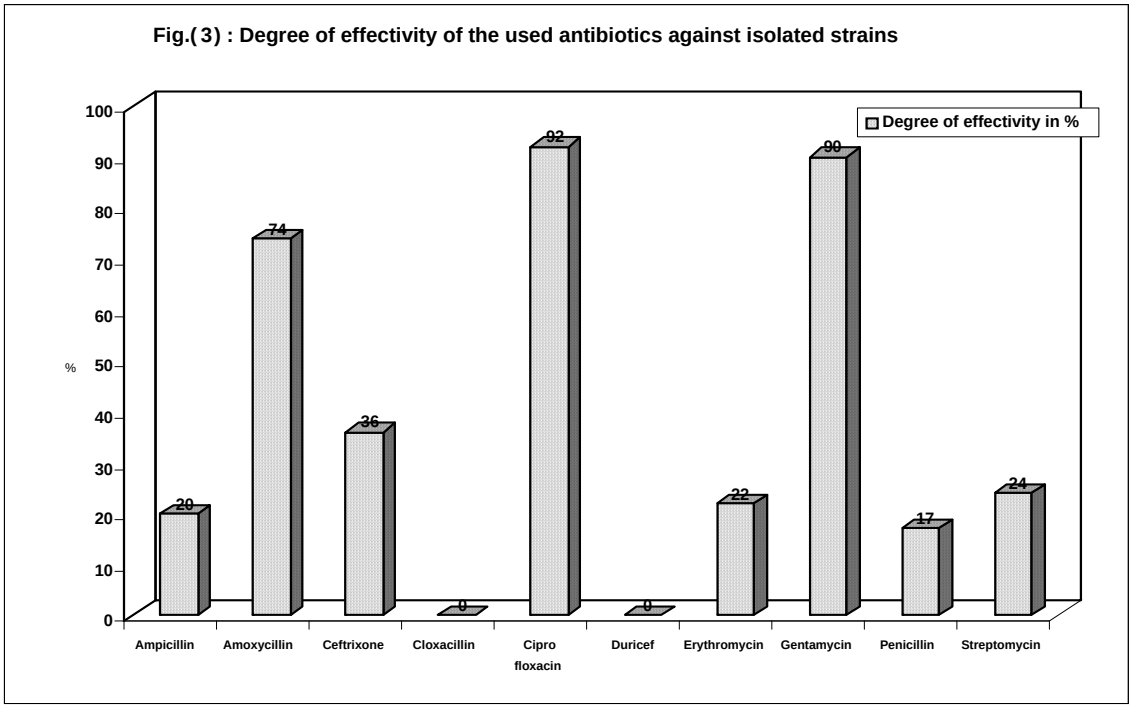


Fig.(3) : Degree of effectivity of the used antibiotics against isolated strains
Degree of effectivity in %
%
0
10
20
30
40
50
60
70
80
90
100
20
74
36
0
92
0
22
90
17
24
Ampicillin
Amoxycillin
Ceftrixone
Cloxacillin
Cipro floxacin
Duricef
Erythromycin
Gentamycin
Penicillin
Streptomycin

DISCUSSION :

Endometritis is one of the major gynecological problems and among infectious causes of infertility in cattle, it ranks first both in heifers and cows (Anjaneyulu, *et al.,* 1999)

The present study was designed to determine the bacteria which are present in vaginal discharges in cases of endometritis and also the normal bacterial flora in different reproductive stages of cattle life.

Through the routine gynecological examination of animals in a Friesian cattle farm in Assiut Governorate, six cows were recorded with endometritis (group 1) became repeat breeder, these cows were culturally positive for bacterial isolation (Table 1 & Fig. 1). Out of the six cows 5 (83.33%) showed mixed infection while the sixth (16.66%) showed a single infection; the high percentage of mixed infections would reflect to some extent the weak immune status of those cows to catch infection and establish the disease. Such mixed infections were also recorded by Dholakia, *et al.,*(1987).

From table 2 and Fig. 2, it is shown that the total isolates in the first group were 11 strains. These bacteria were 3 (27.27%) Staph. sp, 1 (9.09%) Strept. sp. and 7 (63.63%) Corynebacterium sp. It is evident that the main cause of endometritis in the first group was Corynebacterium sp. and this result came in agreement with the results of several workers (Diker, *et al.*1989; Khan, *et al.* 1990; Riberio *et al.* 1990; Takacs, *et al.,* 1990; Bonnett, *et al.*1991; Singla, *et al.,* 1993 and Krishnan *et al.,* 1994).

As regards the 3 strains of Staph. sp. isolated in cases of endometritis, 2 strains were *S.saprophyticus* and one strain was *S.epidermidis* (Table 3) on the basis of polymyxin sensitivity scheme (Quenn, *et al.,* 1994). However this organism is usually present in healthy conditions but in cases of low resistance it became pathogenic so it must not be neglected in microbiological evaluation of such infected cases. The isolation of Staphylococcus sp. from similar conditions by some workers (Riberio, *et al.*, 1990; Kudryavtsev, *et al.,* 1991; Osman, *et al.,* 1991 and Krishnan, *et al.*1994).

Diplococcus (*S. pneumoniae)* was isolated only in one case of endometritis mixed with Corynebacterium sp. in the present work. Streptococcal infection as a non-specific infection in endometritis was also observed by a good number of authors (Khan, *et al.* 1990; Takacs *et al.,* 1990; Biolatti *et al.*1991; Bonnett, *et al.,*1991; Kudryavtsev *et al.* 1991; Osman *et al.,* 1991 and Krishnan *et al.,* 1994).

As regards the second group of pregnant heifers, 12 (92.30%) out of 13 were culturally positive for bacterial isolation. Of these positives, 3 (25%) showed single infection while 9 (75.0%) showed mixed infection and 21 bacterial strains could be isolated (table 1 & Fig.1). These findings reflect the frequency of mixed infection as well as the large numbers of bacterial strains involved in relation to the number of animals examined.This may be attributed to the stress factors of pregnancy when the animal is for a long time under the influence of progesterone (Arthur, 1975), which gives a good chance for the bacterial flora to grow, multiply and attack the tissues in its vicinity.

The most predominant bacterial isolates in the second group were members of family Enterobacteriaceae (Table 2& Fig. 2) which represent 66.66% (14 strains). The isolates included 12 strains of untypable *E.coli*, 1 strain of *Enterobacter aerogenes* and 1 strain of *Klebsiella oxytoca*. In general, these bacteria were also isolated by Carmona *et al.*, (1993) and Krishnan *et al.*, (1994). In addition to the

members of Enterobacteriaceae, 4 (19.4%) strains of *S.epidermidis* and 3 (12.28%) strains of *Corynebacterium sp.* were isolated in the second group of pregnant heifers.

As regards the third group of non pregnant heifers (Table 1& Fig.1) all the 12 examined animals were culturally positive for bacterial isolation and the total isolates were 12 strains and no mixed infection was observed in this group. Table 2 and fig.2 showed that the isolated bacteria were 8 (66.66%) anthracoid bacilli, 3 (25%) *S.epidermidis* and 1 (8.33%) untypable *E.coli*. These isolates seemed to be normal inhabitants in the vaginal secretion, especially those heifers which have not been introduced to mating yet which may a method of infection transmission. On other hand, Carmona *et al.*(1993) mentioned that the differences of the microflora between clinically healthy and sick cows, between cows with normal or abnormal deliveries or between cows and heifers were not significant.

For the fourth group (5 primiparus cows), only 3 (60%) cows showed positive culture was of the mixed type of isolation (table 1 & fig.1). The isolated bacteria were 3 (50%) *S. epidermidis* and 3 (50%) *Enterobacteriacea* which included 2 strains of untypable *E.coli* and one strain of *Citrobacter diversus* (Table 2 fig. 2). The results are in agreement with those recorded by Takacs *et al.,* (1990); Osman *et al.,* (1991) and Krishnan *et al.,* (1994).

Table (3) showed the total number of each species isolated in the present study. The isolated strains were subjected to antibiogram sensitivity testing (table 4 & Fig. 3). It was found that ciprofloxacin and gentamycin were the best of the used antibiotics, since each gave a sensitivity of 92% and 90% respectively. The results are in accordance with those observed by many workers (Kalorey *et al.,* 1983; Rahman and Baxi, 1983 b; Rajangam *et al.,*1989; Krishnan, 1994 and Manohar Paul and Venkatesan 1995).

The least effective antibiotics were cloxacillin and duricef to which all the microorganisms showed no sensitivity. This behaviour may be attributed to the wide misused antibiotics which would lead finally to the development of resistant strains. The resistance to duricef coincides with that recorded by Abd El-Hafeez *et al.,* (2001). To the other antibiotics such as penicillin, ampicillin, erthromycin, streptomycin and ceftrixone, the isolated organisms showed variable degrees of weak sensitivity viz 17%, 20%, 22%, 34% and 36% respectively. This may be also attributed to that such antibiotic was used for a long ago then consequently microbial resistance developed.

In conclusion, repeat breeder cows due to bacterial infection will constitute a serious problem causing great economic losses as well as low fertility of cows, and therefore great efforts must be done to overcome this hazard. Such efforts include medical care of dams during pregnancy period, at parturition and during post-partum period to avoid contamination of the genital system. Appropriate antibiotics must be used, according to the results of sensitivity testing, to avoid high cost and developed antibiotic resistance.

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التقييم البكتريولوجى للافرازات المهبلية للأبقار المصابة بالتهابات رحمية والعجلات السليمة ظاهريا بمحافظة أسيوط

الدكتور/ حسين على عبد القادر * ، الدكتور/ شحاتة حسن شحاتة**

*** معهد بحوث صحة الحيوان – أسيوط**

**** قسم التوليد والتلقيح الاصطناعى – كلية الطب البيطرى – جامعة أسيوط**

أجريت هذه الدراسة بغرض عزل وتصنيف البكتيريا المصاحبة للافرازات المهبلية لعدد ٣٦ بقرة- بعد إجراء الفحص التناسلى- من أحد مزارع الأبقار الفريزيان بمحافظة أسيوط ، وذلك فى الظروف المرضية والصحية العادية ، وصنفت هذه الأبقار إلى أربعة مجموعات : المجموعة الأولى : اشتملت ٦ بقرات متكررة الشيوع مصابة بالتهابات رحمية بعد الولادة، المجموعة الثانية: اشتملت ١٣ عجلة عشار سليمة إكلينيكيا ، المجموعة الثالثة : اشتملت على عدد ١٢ عجلة نامية فى عمر التعشير ، والمجموعة الرابعة والأخيرة اشتملت على عدد ٥ بقرات فى أول ولادة لها.

وقد أسفرت الدراسة عن عزل وتصنيف عدد ٥٠ عترة بكتيرية من الافرازات المهبلية صنفت كالآتى: ١٥(٣٠%) عترة من الميكروب القولونى وقد صنف سيرولوجيا ، ١١(٢٢%) من الميكروب المكور العنقودى الابيدرميدس، ٢(٤%) من الميكروب المكور العنقودى السابروفيتكس، ١(٢%) من الميكروب المتكور السبحى النيمونى ، ١٠(٢٠%) من ميكروب الكورينى ، ٨(١٦%) من الانثراكويد ، ١(٢%) ميكروب الانتيروباكتر ايروجينز ، ١(٢%) الكلبسيلا اوكسى توكا ، الستروباكتر دايفرسيس .

وقد أجريت اختبارات الحساسية لهذه الميكروبات المعزولة باستعمال عشرة أنواع من المضادات الحيوية المختلفة ، وقد وجد أن أفضل هذه المضادات الحيوية المستعملة كان السبروفلوكساسين بدرجة حساسية ٩٢% يليه الجنتاميسين بدرجة حساسية ٩٠% يليه الاموكسيسيلين بدرجة حساسية ٧٤% بينما لم يظهر الكلوكساسيلين أو الديورسيف أى حساسية على الإطلاق (٠%) ، وقد نوقشت الأهمية الصحية لهذه الميكروبات المعزولة .