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THE EFFECT OF USING ANTIMYCOTIC DRUGS UPON THE LIVABILITY OF MYCOTOXIC MOLDS AND PATHOGENIC YEASTS

(With 2 Tables and 4 Figures)

By

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تأثير بعض العقاقير المضادة للفطريات على الفطريات المفرزة للسموم وبعض الخمائر المرضية

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تم جمع ١٢ عينة من الأجنة المجهضة وكذلك ٤٨ عينة من اللبن من حيوانات تعانى من التهاب الضرع وكذلك ١٦ عينة من السائل المنوى المجمد ثم فحصت جميعها فطريا . وقد تم عزل ١٦٣ عينة من الفطريات وكذلك الخمائر وهى تشمل مجموعة الأسبرجلس [فلافس - نيجر - اكراسس]. وكذلك الفيوزاريم والبنسليم والميوكر والكلادوسبوريم والكنديدا ألبيكانس. وقد تم اختيار ٩٥ عينة من أكثر الفطريات سمية وكذلك الخمائر المرضية ووضعت تحت الاختبار ضد سبعة من مضادات الفطريات المختلفة بتركيزات مختلفة . وكانت أكثر الفطريات سمية [مجموعة أسبرجلس] حساسة لكثير من مضادات الفطريات وكان أكثر هذه المضادات تأثيرا هو لاميزيل بالتركيز الأقل . أما عن بعض مضادات الفطريات وهى جريزوفولفين و ميوكوستات والفولفين فكان لها تأثير ضعيف على معظم الفطريات السامة . وعند استخدام اللاميزيل فى علاج بعض حالات التهاب الضرع الناتج عن الاصابة الفطرية كان له تأثير فعال مع الشفاء الكامل للضرع .

SUMMARY

12 samples of aborted foeti, 48 samples of mastitic milk and 16 samples of straw semen were examined for presence of molds and pathogenic yeasts. 163 strains of mold and yeast were isolated. These included Aspergillus spp. (Flavus, niger, achraceus), Fusarium spp., Penicellium spp., Mucor spp., Cladosporium spp. and Candida albicans. 95 strains of most toxigenic molds and pathogenic yeast (Asp. flavus, Asp. achraceus, Fusarium spp., Penicellium spp. and Candida albicans) were tested against 7 antimycotic drugs with different concentrations (30 µg and 10 µg). The predominant

toxigenic fungi (Asp. spp.) were sensitive to a wide range of antimycotic drugs. The most effective antimycotic agent was Lamisil with low concentration 10 µg. Griseofulvin, mucostate and fulvin had a very little effect. In using lamisil in treatment of some cases of mycotic mastitis, it was very effective with complete recovery.

***Key words** : Molds and yeasts - Livability - Antimycotic Drugs*

INTRODUCTION

The indiscriminate use of broad spectrum antibiotics and corticosteroids has led to an increase in the incidence of fungal infections (Huppert et al., 1953).

Fungal infections of the bovine genital tract resulting in infertility have been described by several investigators. It has been found to be the commonest cause of diagnosed abortions in some years. The main cause were species of Aspergillus, Fusarium, Mucor, Penicillium and Candida (Sinha et al., 1980; Mishra et al., 1984; Singh et al., 1991).

The incidence of mycotic mastitis appears to be on the increase because of extensive and rather indiscriminate use of antibiotics for treatment of mastitis. Mycotic mastitis in the cow occurs where antibiotic therapy is used without sufficient precaution under poor hygienic conditions. The main cause was also species of Aspergillus, Mucor and Penicillium. The yeast is not very common but at the same time is not an unusual causative agent in bovine mastitis and yeast infections contribute to the importance of this problem (Johnston et al., 1983).

With the wide spread of artificial insemination, the rate of contamination of semen with different microorganisms become very important. Many kinds of bacteria grow in semen during storage. The addition of antibiotics to semen extenders prevent bacterial growth, but they have no effect on fungi or yeast contaminated semen (Almquist et al., 1949).

The present study was undertaken to establish the effect of using of some antimycotic drugs upon the livability of mycotoxic molds, pathogenic yeasts responsible for reproductive problems, mastitis and contaminated semen.

MATERIAL AND METHODS

Handling of aborted bovine foeti :

Tissue samples from liver, heart, lung and kidney were examined mycologically in Mycology Lab., Animal Reproduction Research Institute, for the presence of different toxigenic molds and pathogenic yeasts.

One gram from each liver, heart, lung and kidney was weighed. Every tissue sample was haemogenized with 3 ml of distilled water in tissue haemogenizer, model no. SDT serial no. 89530. After thorough mixing of tissue samples haemogenate, duplicate petri dishes were inoculated each with 1 ml from tissue haemogenate and plated using about 15 ml quantities from melted and tempered sabouraud dextrose agar (oxide). The inoculated plates were left to solidify at room temperature, then inoculated at 25°C and 37°C for 7 days. Isolation, purification and identification of the isolates was done according to (Paper and Fennell, 1965; Samson, 1979).

Handling of semen samples :

The straw frozen semen samples were examined mycologically in Mycology Lab., Animal Reproduction Research Institute, for the presence of different molds and pathogenic yeasts.

Each straw was removed from liquid nitrogen (-196°c) and dipped into water bath (37°C) for thawing up to 10 minutes, then a loopful from undiluted semen was inoculated on the specific selective media (sabouraud dextrose agar). Isolation, purification of the isolates was applied (Culvenor, 1974; Hajsing and Kopljar, 1964; Hajsing et al., 1962).

Handling of milk samples :

The milk samples were taken from cows with clinical mastitis. About 10-15 mls of milk were aseptically collected into sterile universal bottles, collection was done early in the morning and the samples were transported the same day in ice boxes to the Department of Mycology Lab., Animal Reproduction Research Institute, for mycological examination. A loopful from each sample was inoculated on the specific selective media. Isolation and purification of the isolates was done according to (Cowan and Steel, 1973).

Antimycotic sensitivity tests :

Antimycotic sensitivity testing was carried out on 68 strains of Aspergillus flavus, 3 strains of A. achraceus, 9 strains of Fusarium, 5 strains of Penicillium and 10 strains of Candid albicans using oxoid multo disks Code 1789 E, containing Lamisil (10 and 30 µg), Diflocan (10 and 30 µg), Sporonox (30 µg), Mizoral (30 µg), Griseofulvin (30 µg) and Fulvin (30µg).

Plates were incubated at 25°C and the diameter of the zone of inhibition was usually measured after 5 days of incubation. Inhibition zones were interpreted in accordance with the criteria listed in Table (2) which are based on the recommendation of Blair et al. (1970).

Treatment

Seven cows with mycotic mastitis were used for the study. Quarters were efficiently selected for inoculation. One or two affected quarters of each cow were inoculated. If 2 were inoculated, they were usually inoculated on the same day. Inoculum was prepared by emulsefing lamisil tablet in one ml of distilled water and the appropriate emulsion was injected into each quarter via the teat canal. Each quarter was inoculated immediately following the evening milking. Treatment were continued for all quarters for 8 weeks. A quarter was considered to be cured when the yeast and molds were not isolated from samples taken at 2 successive milkings.

RESULTS

12 samples of aborted foeti, 48 samples of mastitic milk and 16 samples of straw semen were examined for presence of molds and yeasts. Out of which 68 (41.7 %) strains of aspergillus flavus were isolated (Table 1): 41 (25.2 %) strains of A. niger. 3 (1.8 %) strains of A. achraceus. 9 (5.5 %) strains of Fusarium. 5 (3.0 %) strains of Penicillium. 24 (14.7 %) strains of Mucor. 3 (1.8 %) strains of Cladosporium. 10 (6.1 %) strains of Candida.

Table (2) records the relative resistance of the isolated fungi to inhibition by antimycotics. The index of resistance represents the proportion of antimycotics tested to which each strain was mostly resistant.

Most of Aspergillus flavus strains were sensitive to Lamisil, Nizoral, Diflocan and Sporonox. They were resistance to griseofulvin, fulvin and mucostate.

Aspergillus achraceus strains were sensitive to lamisil nizoral, diflocan and sporonox. They were resistance to griseofulvin, mucostate and fulvin.

Fusarium strains were sensitive mostly to diflocan, sporonox, nizoral and lamisil. They were resistance to griseofulvin, mucostate and fulvin.

Penicillium strains were sensitive mostly to lamisil sporonox, nizoral and diflocan. They were resistance to griseofulvin, fulvin and mucostate.

Candida albicans were sensitive mostly to diflocan, nizoral, sporonox and lamisil. They were resistance to griseofulvin, mucostate and fulvin.

Non of the isolated fungi tested was resistant to all antimycotic drugs.

DISCUSSION

In the present study, Aspergillus species were the most frequently encountered and made up 112 of the 163 isolates (68.7 %) (Table 1). This means that A. species were the main fungi responsible for reproductive disorders, clinical mastitis or contaminated semen, and this were on the opposite of Topalka and Furnsnorth (1968) and Farnsworth and Sorenson (1972) who reported that candida species were most frequently encountered. Fusarium species and Penicillium species were encountered too and isolated from (5.5%) and (3.0%) of cases respectively. Mucor 24 (14.7 %), cladosporium 3 (1.8 %) and candida albicans 10 (6.1 %). This percentage of candida albicans was considered a low percentage compared with Sarma and Boro (1980) who had higher frequency of (13.3 %).

In Table (2), the resistance of most of the isolates to griseofulvin and fulvin is probably due to the indiscriminate use of these drugs for the treatment of mycotic troubles. The aspergillus flavus isolates were resistant to griseofulvin and fulvin (Johnston et al., 1983).

It is important to drew attention to the possible health hazards to humans consuming raw milk containing antimycotic resistance Aspergillus flavus, organisms as they may transfer their resistance to other pathogenic organisms present in the intestinal tract of the consumer. Aspergillus achraceus, Fusarium species, Penicillium species, and Candida species were also resistance to both griseofulvin and fulvin.

From the results of the sensitivity test, it is recommended that intra mammary preparations containing lamisil or diflocan or sporonox or nizoral could be used for blind treatment of mycotic mastitis or reproductive problems. They could be added to antibiotics in preparation of straw semen, further trials should be carried out with a view to including these antimycotic drugs in extendor on a routine basis.

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Table 1: Fungi isolated from aborted foeti, clinical mastitic cases and contaminated semen.

Fungi isolated	Aborted foeti n = 12		Milk samples n = 48		Contaminated semen (n=16)		Total	% relative frequency
	No.	Frequency	No.	Frequency	No.	Frequency		
A. Flavus	28	52.8	22	34.3	18	39.1	68	41.7
A. niger	12	22.6	15	23.4	14	30.4	41	25.2
H. achraceus	3	5.7	-	-	-	-	3	1.8
Fusarium spp.	2	3.8	4	6.3	3	6.5	9	5.5
Penicillium	-	-	3	4.7	2	4.3	5	3.0
Mucor spp.	5	9.4	16	25.0	3	6.5	24	14.7
Cladosporium	-	-	2	3.1	1	2.2	3	1.8
Candida albicans	3	5.7	2	3.1	5	10.9	10	6.1
Total	53	100	64	100	46	100	163	100

Table 2: Drug sensitivity of 95 of 163 strains of mold and yeast isolated from aborted foeti,, milk samples and contaminated semen.

Fungi isolated	Total No. isolated	Ni S/R	Sp. S/R	La. S/R	Di. S/R	Gr. S/R	Mu. S/R	Ful. S/R
A. Flavus	68	64/4	53/15	68/0	61/7	2/66	1/67	2/66
A. achraceus	3	3/0	2/1	3/0	2/1	1/2	0/3	0/3
Fusarium	9	5/4	6/3	5/4	8/1	0/9	0/9	1/8
Penicillium	5	4/1	5/0	5/0	4/1	0/5	1/4	0/5
Candida	10	8/2	6/4	6/4	10/0	2/8	1/9	0/10

S/R = Sensitive/Resistant Ni = Nizoral Sp. = Sporonox
La. = Lamisil Di = Diflocan Gr. = Griseofulvin
Mu. = Mucostate Ful. = Fulvin.

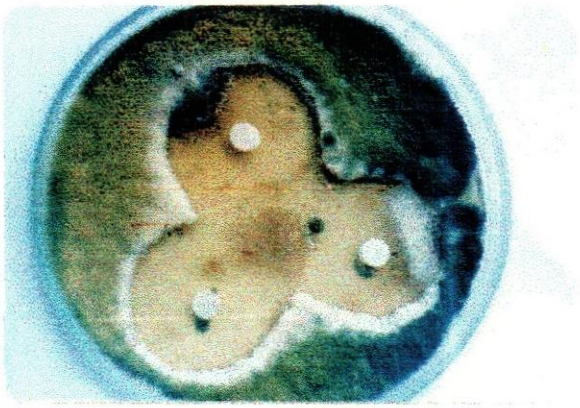

Fig. 1: The plate represent pure aspergillus flavus strain with a wide clear zone of inhibition with lamisil discs 10 µg (low concentration).

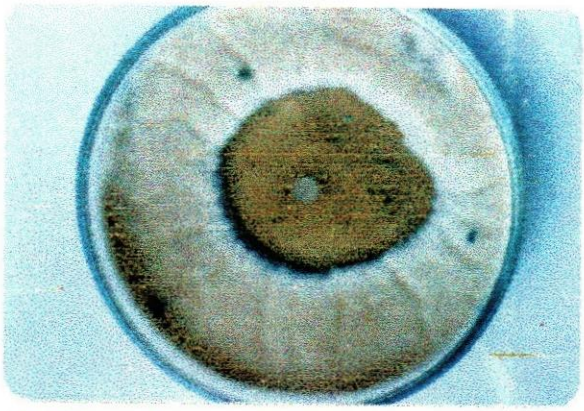

Fig. 2: The plate represent pure fusarium strain with a wide clear zone of inhibition with lamisil discs 10 µg (low concentration).

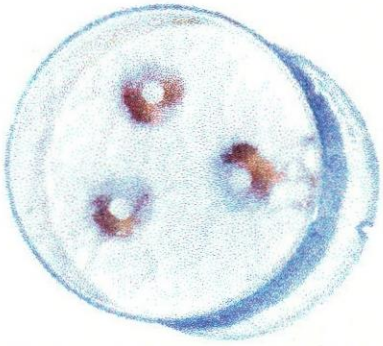

Fig. 3: The plate represnt pure aspergillus flavus strain with a wide clear zone of inhibition with sporonox 30 µg (high concentration).

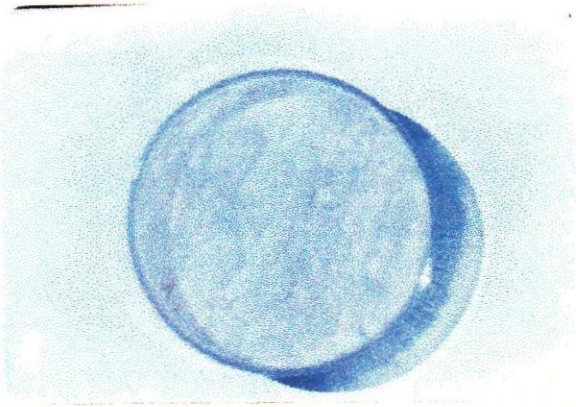

Fig. 4: The plate represent pure fusarium strain with no zone of inhibition with fulvin 30 µg (high concentration).