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Antifungal susceptibility of *Candida* spp isolated from bovine mammary glands and teat cups of milking machines

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*Candida*속 균의 항진균성약제에 대한 감수성

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초록 : 준임상형 유방염 이환 젖소의 유즙과 유두컵으로부터 분리된 *Candida*속 균의 8종 항진균성약제에 대한 감수성을 조사하였던 결과는 다음과 같다. 총 53주의 *Candida*속균 중 98.2%, 94.4%, 84.8%의 균주가 각각 clotrimazole, econazole, miconazole의 농도 25μg/ml이하에서 발육이 억제됨으로서, 이들 약제의 항균력이 타 약제에 비하여 우수하였다.

균종별로 보면 *C albicans*는 clotrimazole에 대한 감수성이 가장 높았으며, 기타 비교적 감수성이 높았던 약제는 5-fluorocytosine, econazole, miconazole의 순이었다. *C pseudotropicalis*와 *C guilliermondii*는 haloprogin, clotrimazole, miconazole, econazole, 5-fluorocytosine등에 높은 감수성을 나타내었으며, haloprogin의 평균 MIC가 0.17~0.19μg/ml로 가장 우수하였다. *C krusei*는 cycloheximide에 대한 감수성이 가장 높았으며, 그의 비교적 감수성이 높았던 약제는 clotrimazole, haloprogin, miconazole, econazole의 순이었다.

한편 *C parapsilosis*는 econazole, cycloheximide, clotrimazole등에 다소 감수성을 나타내었으나, 이들 약제의 MIC는 econazole(기하평균 MIC 7.26μg/ml)을 제외하고는 타균종에서 보다 현저히 낮았다. 또한 *C tropicalis*는 전 공시약제에 대하여 감수성이 낮은 경향이었다.

Key words: cow, candida, antifungals, susceptibility, mastitis

Introduction

Since bovine mastitis is a major cause of economic loss to the dairy farm, numerous control programs are indicated in accordance with the pathological changes of the mammary glands and inducing microorganisms.[1] Determination of antimicrobial susceptibility of the causative organism is also practised prior to the treatment. These were, however, the treatment regimens mainly for the eradication of bacterial mastitis.

Treatment of yeast-induced mastitis has in general been unsuccessful because selection of antimicrobial drugs is rather limited. Some investigations[2-4] revealed that clinical mastitis caused by the species of *Candida*, *Cryptococcus*, *Trichosporon* and *Torulopsis* were treated by intramammary infusion of nystatin, clotrimazole or undecylenic acid. Recently, interest concerning to the control program of yeast-induced mastitis is to broaden the clinical use of effective drugs.

The purpose of the present study was to determine

the in vitro susceptibility of *Candida* spp to antifungal drugs.

Materials and Methods

Test strains: The 53 strains of *Candida* spp tested were isolates from milk of dairy cows with subclinical mastitis and teat cups of milking machines. The strains were identified as 5 *C albicans*, 10 *C tropicalis*, 17 *C pseudotropicalis*, 5 *C parapsilosis*, 7 *C krusei* and 9 *C guilliermondii* in other paper. All strains were maintained on Sabouraud's dextrose agar(SDA) before use.

Preparation of antifungal drugs and test medium: The antifungal drugs tested were nystatin(5640 units/mg), clotrimazole, miconazole, econazole, 5-fluorocytosine, cycloheximide, haloprogin and griseofulvin(Sigma Chemical Co, USA). According to the agar dilution method of Bryant[5] and Bergan and Vangdal[6], the individual drug was dissolved in 10ml of absolute methyl alcohol. The drug solution was then diluted by serial twofold method with 10ml of sterile distilled water. Afterwards, the drug solution was added to 90ml of SDA which was sterilized and cooled to 40~45°C before. After mixing thoroughly, the medium was poured onto Petri dish to solidify. The final concentrations of the drugs in test medium were ranged from 0.05 to 200μg/ml.

Susceptibility test: Antifungal susceptibility test was done by the methods of Bryant[5], Bergan and Vangdal[6], and Yamaguchi et al.[7] Overnight cultures of test strains in Sabouraud's dextrose broth were diluted with sterile buffered saline to match the turbidity of standard $BaSO_4$ solution.[8] The approximate cell density was 1.5×10^8/ml. These were inoculated onto antifungal media with the multiple inoculator.[9] Results were read after 24 hours' incubation at 37°C, and the minimum inhibitory concentration(MIC) was defined as the lowest concentration of drugs completely inhibiting fungal growth. Growth was abundant at that time on the drug-free control plates. MIC values were explained as geometric mean (GM) in certain case of result evaluation.

Results

Antifungal susceptibilities in 53 strains of the genus *Candida* were shown in Table 1. The 98.2% of the *Candida* strains were inhibited by clotrimazole at ≦25μg/ml. In order of antifungal activity at≦25μg/

Table 1. Susceptibilities of 53 strains of *Candida* spp to 8 antifungal drugs

MIC* (μg/ml)	Cumulative percentage of strains inhibited by							
	Nystatin	Cycloheximide	5-Fluorocytosine	Griseofulvin	Clotrimazole	Miconazole	Econazole	Haloprogin
<0.05	1.9	0	1.9	0	0	0	0	5.7
0.05	1.9	0	1.9	0	0	0	0	5.7
0.1	1.9	0	1.9	0	0	0	0	28.3
0.2	1.9	1.9	3.8	0	1.9	0	0	39.6
0.4	1.9	5.7	3.8	0	3.8	1.9	0	41.5
0.8	1.9	17.0	3.8	1.9	22.7	3.8	0	47.2
1.6	1.9	18.9	5.7	3.8	32.1	3.8	5.7	56.6
3.2	1.9	26.4	13.2	3.8	64.2	11.3	43.4	69.8
6.3	1.9	30.2	35.8	3.8	69.9	62.2	49.1	69.8
12.5	13.2	34.0	43.3	3.8	73.7	62.2	77.4	69.8
25	43.4	35.9	71.6	3.8	98.2	84.8	94.4	69.8
50	81.1	35.9	96.1	3.8	98.2	86.7	94.4	69.8
100	96.2	35.9	96.1	5.7	98.2	92.4	94.4	73.6
200	100.0	49.1	96.1	18.9	98.2	94.3	94.4	77.4
>200	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

* MIC: minimum inhibitory concentration.

Table 2. Geometric mean values of minimum inhibitory concentration (μg/ml) of antifungal drugs against 6 different species of *Candida*

Species	No of Strains	Nystatin	Cycloheximide	5-Fluorocytosine	Griseofulvin	Clotrimazole	Miconazole	Econazole	Haloprogin
C albicans	5	37.90	76.13	9.52	132.60	5.49	16.65	9.56	43.94
C tropicalis	10	46.65	229.80	75.78	400.00	20.32	57.44	28.81	187.10
C pseudotropicalis	17	14.15	96.54	5.59	266.40	1.96	4.76	5.17	0.17
C parapsilosis	5	37.90	11.02	28.71	400.00	12.54	25.00	7.26	66.27
C krusei	7	22.64	0.54	20.53	362.30	0.80	6.30	12.50	2.15
C guilliermondii	9	67.96	252.00	5.40	233.20	3.20	6.30	3.45	0.19

ml, followed were econazole and miconazole which inhibit respectively 94.4% and 84.8% of the strains. The 3.8% to 71.6% of tested strains were inhibited by griseofulvin, cycloheximide, nystatin, haloprogin and 5-fluorocytosine at≦25μg/ml.

Susceptibilities of 6 different species of *Candida* to 8 antifungal drugs were compared in Table 2. *C albicans* was most sensitive to clotrimazole of which GM-MIC was 5.49μg/ml and relatively sensitive to 5-fluorocytosine, econazole and miconazole showing GM-MIC range of 9.52 to 16.65 μg/ml. Whereas, *C albicans* appeared to be less sensitive to other drugs.

C pseudotropicalis and *C guilliermondii* were markedly sensitive to haloprogin, clotrimazole, miconazole, econazole and 5-fluorocytosine. GM-MIC of these drugs was 0.17 to 6.30μg/ml. Haloprogin (GM-MIC, 0.17~0.19μg/ml) was most active. These species were less sensitive to other drugs.

C krusei was highly sensitive to cycloheximide, clotrimazole, haloprogin and miconazole. The GM-MIC of these drugs was 0.54 to 6.30μg/ml. Cycloheximide(GM-MIC, 0.54μg/ml) was most active. This species was relatively sensitive to econazole(GM-MIC, 12.50μg/ml). To other drugs, *C krusei* appeared to be less sensitive.

C parapsilosis was somewhat sensitive to econazole, cycloheximide, clotrimazole and GM-MIC of these drugs was 7.26 to 12.54μg/ml. The sensitivity of *C tropicalis* to all antifungal drugs tested was much low, and GM-MIC of these drugs was over 20.32 μg/ml.

Discussion

Yeast infection is one of the problems sometimes encountered in eradication of bovine mastitis, and it considered to be troublesome disease because most yeasts are resistant to common antibacterial drugs.[10] Though the therapeutic effects of nystatin, clotrimazole and undecylenic acid on yeast-induced mastitis were indicated,[2~4] further investigations on the antifungal susceptibility of yeasts are necessary to extent the clinical use of drugs.

In the present study, the strains of the genus *Candida* were sensitive with notable degree to imidazole antifungals such as clotrimazole, econazole and miconazole(Table 1). The 84.8% to 98.2% of 53 strains were inhibited by these drugs at concentration of≦25μg/ml, and among them clotrimazole was most active. These findings agreed with report of McDonald et al[11] in which 96.7% of yeasts including mostly *Candida* spp of bovine mammary gland origin were sensitive to clotrimazole. Bergan and Vangdal[6] also reported that econazole, miconazole and clotrimazole were most active against *Candida* spp of human origin. From these findings, it would suppose that these imidazole antifungals might be used prior to other drugs for initiation of treatment of bovine mastitis by *Candida* spp.

Interspecies differences of antifungal susceptibility were found in *Candida* spp of the present study (Table 2). *C albicans* was most sensitive to clotrimazole(GM-MIC, 5.49μg/ml) followed by 5-fluorocytosine, econazole and miconazole. These were similar to the findings of Saubolle and Hoeprich[12] in which 66% to 74% of *C albicans* were sensitive to these drugs except nystatin being not tested. Yamaguchi et al[7] also reported that 96% of 27 *C albicans* were inhibited by clotrimazole at≦5μg/ml.

C pseudotropicalis and *C guillermondii* were similar in antifungal sensitivity. They exhibited significant sensitivity to haloprogin, clotrimazole, miconazole, econazole and 5-fluorocytosine. The GM-MIC of haloprogin (0.17~0.19μg/ml) to these species was the most prominent in the present study.

C krusei was notably sensitive to cycloheximide (GM-MIC, 0.54μg/ml) and clotrimazole (GM-MIC, 0.80μg/ml), which agreed with the report of Yeo and Choi.[13] It was known that *C krusei* was also sensitive highly to haloprogin (GM-MIC, 2.15μg/ml in the present study.

C parapsilosis proved to be somewhat sensitive to econazole (GM-MIC, 7.26μg/ml), cycloheximide (GM-MIC, 11.02μg/ml) and clotrimazole (GM-MIC, 12.54μg/ml). The MIC values of these were, however, inferior several times to those in other reports. Bergan and Vangdal[6] showed MIC of econazole, 1.0μg/ml and MIC of clotrimazole, 0.25μg/ml, and Yeo and Choi[13] reported MIC of cycloheximide and clotrimazole, 0.8 to 3.1μg/ml.

C tropicalis showed very low sensitivity to all antifungals in the present study, and even in clotrimazole which was most active, the GM-MIC was 20.32μg/ml. A similar tendency was found by Chang and Kim.[14] They reported that no strains of *C tropicalis* were inhibited by nystatin, cycloheximide, 5-fluorocytosine, griseofulvin and miconazole at concentration of below 25μg/ml.

Summary

In vitro antifungal susceptibility test was carried out on 53 strains of *Candida* spp isolated from milk of dairy cows with subclinical mastitis and teat cups of milking machines. Nystatin, clotrimazole, miconazole, econazole, 5-fluorocytosine, cycloheximide, haloprogin and griseofulvin were tested by the agar dilution method. The 84.8% to 98.2% of *Candida* strains were inhibited by clotrimazole, econazole and miconazole at ≦25μg/ml, and clotrimazole was most active. Interspecies differences of antifungal susceptibility were recognized and these were as follows.

C albicans was most sensitive to clotrimazole (GM-MIC, 5.49μg/ml) followed by 5-fluorocytosine, econazole and miconazole. *C pseudotropicalis* and *C guilliermondii* were notably sensitive to haloprogin, clotrimazole, miconazole, econazole, 5-fluorocytosine, and haloprogin (GM-MIC, 0.17~0.19μg/ml) was most active. *C krusei* was most sensitive to cycloheximide (GM-MIC, 0.54μg/ml) followed by clotrimazole, haloprogin, miconazole and econazole. *C parapsilosis* was somewhat sensitive to econazole, cycloheximide, clotrimazole, and econazole (GM-MIC, 7.26μg/ml) was most active. *C tropicalis* showed very low sensitivity to all tested drugs (GM-MIC, ≧20.32μg/ml).

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