Research Article

Isolation and Identification of Candida spp. from Mastitis Cattle Milk and Determination of Antifungal Susceptibilities

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ABSTRACT

Isolation and identification of Candida spp. and determination of their antifungal susceptibilities that cause mycotic mastitis in cattle in Tekirdag province and its counties in Marmara Region were aimed in this research. 100 mastitis milk samples were used in the study. Microorganisms isolated as Candida spp. were identified by API® 20C AUX rapid identification kit. Antifungal susceptibility rates of identified Candida spp. were determined by using disc diffusion technique. Candida spp. was detected in the 25 of the 100 mastitis milk samples. According to results of API® 20C AUX rapid identification kit, 8 C. krusei (32%), 5 C. albicans (20%), 3 of each C. boidinii, C. famata and C. kefyr (12%), 2 C. spherica (8%) and 1 C. thermophilia (4%) were identified from the 25 isolated strains. In the evaluation of test results of isolated strains that performed in respect to M-44 directive of CLSI (Method for Antifungal Disc Diffusion Susceptibility Testing of Yeasts; Approved Guideline); all strains were found 100% susceptible to Ketoconazole and 100% resistant to Fluconazole, Miconazole, Amphoteracine B and Flucytosine. Two of the C. albicans (40%) and 1 of the C. krusei (12.5%) isolates were susceptible Nystatin while all the other isolates were moderately resistant to that. This study contributes significantly to the literature as one of the rare studies addressing the development of antifungal resistance.

Key words: Mastitis, Candida spp, Identification, Antifungal susceptibility

INTRODUCTION

Mastitis in dairy cattle is an inflammatory reaction of the udder and it is the most common and costly disease in the dairy industry. There are many bacteria known to cause mastitis which include Pseudomonas aeruginosa, Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus agalactiae, Brucella melitensis, Corynebacterium bovis, Mycoplasma spp., Escherichia coli, Klebsiella pneumoniae, Klebsiella oxytoca, Enterobacter aerogenes, Pasteurella spp., Trueperella pyogenes, Proteus spp., and Prototheca spp. (algae). And also several species of yeast or yeast-like microorganisms have been reported to cause bovine mastitis (Krukowski, 2001; Costa et al., 1998).

The incidence of mastitis, due to fungi, is normally very low. The incidence of fungal mastitis in the United States ranges from 2% to 7% (Kirk and Bartlett, 1986). These rates can be higher in tropical climates (Costa et al., 1993). But the incidence has significantly increased during the last decade. Fungus-related mastitis is often caused by Candida spp. Since it is not determined, long-term antibiotic treatments are performed and the development of resistance against many microorganisms is ensured. It is therefore expected that mastitis associated with fungi will pose an important problem in the future (Santos and Marin, 2005).

Every antymycotic agents are not succeeded in treatment of Candida species that are defined generally as opportunistic pathogens. Antifungal medicines that had limited availability before 1980’s have shown increase in usage in the later years. As a matter of fact, rate of rise in fungal diseases over the past decade has been a guide in improvement of antifungal medicines. Growing use of antifungal agents in recent years have suppressed endogenous fungal flora, and more resistant strains were shown up with the suppression of susceptible strains (Koç, 2003).

The aim of the present study was to isolation and identification of Candida spp. and determination of their antifungal susceptibilities that cause mycotic mastitis in cattle in Tekirdag province and its counties in Marmara Region/Turkey.
MATERIALS AND METHODS

Sample collection
In this research, 100 mastitic milk samples were used that were collected from the farms of the Tekirdag region of Turkey by performing California Mastitis Test (CMT). The procedures have been described previously (Quinn et al., 1994). For collections of milk samples, the teat tips were cleaned by using 70% alcohol impregnated swabs and allowed to dry. After discarding the first few milk jets, 2-5 ml of the milk samples were collected into sterile glass flasks. Then they were brought to Adnan Menderes University Faculty of Veterinary Medicine Department of Microbiology Laboratory under the cold chain.

Isolation and identification of Candida spp.
Milk samples were cultured on 4% Sabouraud Dextrose Agar (SDA) (Merck 1.05438) and were incubated separately at 25-37°C. The growths were checked on a daily basis and after 72 hours, the samples without yeasts were excluded from the study. From the colonies that grown in SDA in 2-3 days, in paste stiffness, 0.5-1 mm in diameter, white or cream colored, uniformly bounded and that have distinctive yeast scent were subjected to the gram staining.

In Gram staining, Gram positive, oval or prolonged budding yeast cells, individual, sometimes double, triple blastospor clusters and pseudohyphogenic yeast cells isolated as Candida spp. Under the gram staining evaluation, microorganisms that were predefined as Candida spp. were conveyed to SDA and pure cultures were obtained (Bilgehan, 1995). All the pure cultures were passaged to the slanting SDA and freezeed in -20°C until usage.

Finally, pure isolates were identified by API® 20C AUX (BioMerieux, France) rapid identification kit according to the manufacturer’s instructions.

Antifungal susceptibility tests
Antifungal susceptibility tests were performed for Candida spp. isolates by using the M-44 directive of CLSI (Method for Antifungal Disc Diffusion Susceptibility Testing of Yeasts; Approved Guideline) (CLSI, 2009). The antifungal discs used for tests included ketoconazole [10 µg], fluconazole [10 µg], miconazole [10 µg], amphotericin B [20 µg], nystatin [100 IU] and fluocytosine [1 µg].

RESULTS

Isolation and identification results
In this study, 25 Candida spp. were detected in the 100 mastitic milk samples. By using API® 20C AUX (BioMerieux, France) rapid identification kit, 8 C. krusei (32%), 5 C. albicans (20%), 3 each of C. boidinii, C. famata and C. kefyr (12%), 2 C. sphaeria (8%) and 1 C. thermophila (4%) were identified from the 25 isolated strains (Table1).

Antifungal susceptibility tests results
In the evaluation of the test results of isolated strains that performed as in M-44 directive of CLSI (Method for Antifungal Disc Diffusion Susceptibility Testing of Yeasts; Approved Guideline): all strains were found 100% susceptible to Ketoconazole and 100% resistant to Fluconazole, Miconazole, Amphotericine B and Fluocytosine. Two of the C. albicans (40%) and 1 of the C. krusei (12.5%) isolates were susceptible to Nystatin while all the other isolates were moderately resistant to that (Table 2).

Table 1: Identification results of the Candida isolates

<table>
<thead>
<tr>
<th>Candida spp.</th>
<th>Number</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. krusei</td>
<td>8</td>
<td>32</td>
</tr>
<tr>
<td>C. albicans</td>
<td>5</td>
<td>20</td>
</tr>
<tr>
<td>C. boidinii</td>
<td>3</td>
<td>12</td>
</tr>
<tr>
<td>C. famata</td>
<td>3</td>
<td>12</td>
</tr>
<tr>
<td>C. kefyr</td>
<td>3</td>
<td>12</td>
</tr>
<tr>
<td>C. sphaeria</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>C. thermophila</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Total (n=25)</td>
<td></td>
<td>100</td>
</tr>
</tbody>
</table>

DISCUSSION

The isolation, identification and determination of antifungal susceptibility of Candida spp. that cause mycotic mastitis in cattle in Tekirdag province and its counties in Marmara Region/Turkey were investigated in this study. Twenty-five isolates belonging to Candida genus cultured from 100 milk samples were examined. Our investigation shows a high frequency of Candida spp. infections, approximately 25% of all examined samples. This result is pretty high when compared with the other studies (Awad et al., 1980; Krukowskki et al., 2001; Tel et al., 2009, Yesilmens et al., 2012). Presence of Candida spp. in mastitic milks show differences in various parts of the world in example 6.1% in Egypt (Awad et al., 1980), 1.3% in Denmark (Aalbek et al., 1994) and 12.07% in Brazil (Costa et al., 1993). Higher Candida spp. percentage (25%) obtained in our research was attributed to focusing the sampling persistent mastitis cases that have long term antibiotic treatments.

Distribution of Candida species shows diversity in several surveys, according to some researchers C. krusei, C. rugosa, and C. albicans have been demonstrated as the most common species isolated from mycotic mastitis (Costa et al., 1993; Santos and Marin, 2005; Aalbek et al., 1994). In the identification of the 45 Candida species obtained in the study of Santos and Marin (2005), most frequently isolated ones were C. krusei (44.5%), C. rugosa (24.5%), C. albicans (8.9%) and C. guilliermondii (8.9%). Krukowskki et al.(2006) reported that the most frequently isolated species were C. kefyr, C. ciferrii, and C. krusei in the Lublin region (Poland). Also Seker (2010) reported that most frequently isolated species were C. krusei (34.8%), C. rugosa (16.4%), C. kefyr (12.6%), C. albicans (10.1%), and C. tropicalis (9.2%). In our study, most frequently isolated species were C. krusei (32%), C. albicans (20%), C. boidinii, C. famata and C. kefyr (12%), C. sphaeria (8%) and C. thermophila (4%). Variations between studies were derived from the geographical differences.

Some of the Candida species are naturally resistant to some antifungals such as intrinsic fluconazole resistance in C. krusei. It was shown that many Candida isolates have resistance to amphotericin B and some Candida isolates have resistance to ketoconazole (Gunes et al., 105 Inter J Vet Sci, 2017, 6(2): 104-107.
As the resistance to flucytosine in *C. albicans* (10-30%) is known, increasing resistance in non-albicans *Candida* species is begun to mention. Natural or acquired resistance to fluconazole was confirmed in *C. albicans, C. krusei, C. glabrata* and *C. tropicalis* (Arikian, 2002). Full resistance (100%) to fluconazole, Miconazole, Amphotericin B and Flucytosine were determined whereas full susceptibility (100%) to ketoconazole in our study. Two *C. albicans* (20%) and one *C. krusei* (12.5%) were found fully susceptible to nystatin while all the others were medium susceptible.

In the evaluation of results, Nystatin is appeared as second antifungal option following ketoconazole in mastitis cases depending on *Candida*. Identification of *Candida* species is important in choosing medication for this reason (Kusturim, 1999). Increase in infections based on *Candida* species in recent years and various susceptibility to current antifungal agents of these species is showed the importance of invitro susceptibility tests during the decision stage (Uzun, 1998).

**Conclusions**

In conclusion, it is thought *Candida* spp. that are in a high percentage such as 25% in the collected mastitic milks may cause either mastitis or systemic candidiasis with probable decline in the strength of immune system of the animal. In the results obtained from in vitro antymycotic resistance tests for identified species, it is shown that the most effective agent that can be used Ketoconazole. The study also contributes significantly to the literature as one of the rare studies addressing the development of antifungal resistance.

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### REFERENCES


