P-ISSN: 2304-3075; E-ISSN: 2305-4360
International Journal of Veterinary Science
www.ijvets.com; editor@ijvets.com

Research Article

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

Mustafa Sonmez[1] and Goksel Erbas[2*]

[1]Adnan Menderes University, Health Science Institute, TR-09016 Aydin – Turkey
[2]Adnan Menderes University, Faculty of Veterinary Medicine, Department of Microbiology, TR-09016 Aydin – Turkey
***Corresponding author:** gerbas@adu.edu.tr

Article History: Received: April 11, 2017 Revised: April 21, 2017 Accepted: April 26, 2017

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 antimycotic 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, 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 endo-genous 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.

Cite This Article as: Sonmez M and G Erbas, 2017. Isolation and identification of *Candida* spp. from mastitic cattle milk and determination of antifungal susceptibilities. Inter J Vet Sci, 6(2): 104-107. www.ijvets.com

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 freezed 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], amphoteracine B [20 µg], nystatin [100 IU] and flucytosine [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. spherica* (8%) and 1 *C. thermophilia* (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, Amphoteracine B and Flucytosine. 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

Candida spp.	Number	Percentage %
C. krusei	8	32
C. albicans	5	20
C. boidinii	3	12
C. famata	3	12
C. kefyr	3	12
C. spherica	2	8
C. thermophilia	1	4
Total (n=25)	25	100

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; Krukowski *et al*., 2001; Tel *et al*., 2009, Yesilmen *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%). Krukowski *et al*.(2006) reported that the most frequently isolated species were *C. kefyr, C. ciferrii*, and *C. krusei* in the Lublin region (Poland). Also Şeker (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. spherica* (8%) and *C. thermophilia* (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*.,

Table 2: Antifungal suspectibility test results

Candida spp.	ANTIFUNGAL																	
	KCA			MCL			AMB			FCN			NY			FY		
	10 µg			10 µg			20 µg			10 µg			100 Unit			1 µg		
	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
C. krusei (n=8)	8	-	-	-	-	8	-	-	8	-	-	8	1	7	-	-	-	8
C. albicans (n=5)	5	-	-	-	-	5	-	-	5	-	-	5	2	3	-	-	-	5
C. boidinii (n=3)	3	-	-	-	-	3	-	-	3	-	-	3	-	3	-	-	-	3
C. famata (n=3)	3	-	-	-	-	3	-	-	3	-	-	3	-	3	-	-	-	3
C. kefyr (n=3)	3	-	-	-	-	3	-	-	3	-	-	3	-	3	-	-	-	3
C. spherica (n=2)	2	-	-	-	-	2	-	-	2	-	-	2	-	2	-	-	-	2
C. thermophilia (n=1)	1	-	-	-	-	1	-	-	1	-	-	1	-	1	-	-	-	1

S: Suspectible, I: Intermediate, R: Resistant - KCA: Ketoconazole, MCL: Miconozole, AMB: Amphoterycin B, FCN: Fluconazole, NY: Nystatin, FY: Flucytosine.

2001). As the resistance to flucytosine in *C. albicans* (10-30%) is known, increasing resistance in non-albicans *Candida* species is began to mention. Natural or acquired resistance to flukonazole was confirmed in *C. albicans*, *C. krusei*, *C. glabrata* and *C. tropicalis* (Arikan, 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 (Kustimur, 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 antimycotic 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.

Acknowledgments

This study was financially supported by the Scientific Research Fund of Adnan Menderes University by the project number VTF-15069, 2015 and The article is prepared from Mustafa SÖNMEZ's Master of thesis Project 2016, Aydin, Turkey. This Research project has been approved by the Animal Research Ethics Committee of University of Adnan Menderes (64583101/2015/100).

REFERENCES

Aalbaek B, J Stenderup, HE Jensen, J Valbak, B Nylin and A Huda, 1994. Mycotic and algal bovine mastitis in Denmark. APMIS, 102: 451-456.

Arikan S, 2002. The importance of susceptibility tests in the treatment of Candida infections. Candida Microbiology and Infections Symposium (21-22 June 2002, Eskisehir) Congress book. Istanbul, Turkish Microbiology Association, 161-166.

Awad FI, A El Moll, A Fayed, M Abd el-Halim and M Refai, 1980. Studies of mycotic mastitis in Egypt. J Egypt Vet Med Assoc, 40: 35-41.

Bilgehan H, 1995. Clinical Microbiological Diagnosis. Fakulteler Bookstore. Barış Publications Faculties Publishing House, 5. Press, İzmir, pp: 390-400.

CLSI, 2009. Clinical and Laboratory Standards Institute. Method for Antifungal Disk Diffusion Susceptibility Testing of Yeasts; Approved Guideline. CLSI document M44-A2. 2nd ed Wayne.

Costa EO, AR Ribeiro, ET Watanabe and PA Melville, 1998. Infectious bovine mastitis caused by environmental organisms. J of Vet Med B, 45: 65-71.

Costa EO, CR Gandra, MF Pires, SD Coutinho and CM Teixeira, 1993. Survey of bovine mycotic mastitis in dairy herds in the state of Sao Paulo, Brazil. Mycopathologia, 124: 13-17.

Gunes I, A Kalkanci and S Kustimur, 2001. Comparison of Three Different Commercial Kits with Conventional Methods for the Identification of Candida Strains to Species Level. Bull Microbiol, 35: 559-564.

Kirk JH and PC Bartlett, 1986. Bovine mycotic mastitis. Comp Food Anim, 8: 106-110.

Koç N, 2003. Antifungal resistance in our country. 3. National Fungal Diseases and Clinical Micology Congress (27-30 May, 2003) Congress Book. Bornova, Izmir, Turkey, pp: 285-300.

Krukowski H, 2001. Mycotic mastitis in cows. Med Weter, 57: 18-20.

Krukowski H, A Lisowski, P Rozanski and A Skorka, 2006. Yeasts and algae isolated from cows with mastitis in the Southeastern part of Poland. Pol J Vet Sci, 9: 181-184.

Krukowski H, M Tietze, T Majewski and P Rozanski, 2001. Survey of yeast mastitis in dairy herds of small-type farms in the Lublin region, Poland. Mycopathologia, 150: 5-7.

Kustimur S, 1999. Antifungal Suspectibility Tests In: Ustacelebi S. et al. Basic and Clinical Microbiology (1th edition), Gunes bookstore. Ankara pp: 1159-1166.

Quinn PJ, ME Carter, B Markey and GR Carter, 1994. Clinical Veterinary Microbiology. Mosby-Year Book Europe Limited, Lynton House, London, England pp: 40-190.

Santos RC and M Marin, 2005. Isolation of Candida spp. from mastitic bovine milk in Brazil. Mycopathologia, 159: 251-253.

Seker E, 2010. Identification of Candida Species Isolated from Bovine Mastitic Milk and Their In Vitro Hemolytic Activity in Western Turkey. Mycopathologia, 169: 303-308.

Tel OY, O Keskin, AK Zonturlu, and NB Arserim Kaya, 2009. Subclinical Mastitis Prevalance and Determination of The Antibiotics Susceptibility in Sanliurfa Region. F U Sag Bil Vet Derg, 23: 101-106.

Uzun Ö, 1998. Treatment approaches in Fungal Hospital Infections. Turkish J of Hosp Infect 2: 156-163.

Yesilmen S, N Ozyurtlu and S Bademkiran, 2012. The Isolation of Subclinical Mastitis Agents and Determination of the Sensitive Antibiotics in Dairy Cows in Diyarbakir Province. J Faculty Vet Med, University of Dicle, 1: 24-29.