Journal of Veterinary Medicine and Animal Health

Full Length Research Paper

Interaction in the production of biofilm and drug susceptibility of *Candida kefyr* with *Escherichia coli* and *Streptococcus dysgalactiae* isolated from bovine mastitis

Israel Daniel Ricardo-González[1], Laura Hernández-Andrade[2]*, Ana Lilia Del Monte-Rodríguez[2], Marco Antonio Santillán-Flores[2], Miguel Ángel Blanco Ochoa[1], Luis Octavio Campuzano-Reyes[1] and Alberto Jiménez-Saavedra[3]

[1]Facultad de MedicinaVeterinaria y Zootecnia, Universidad NacionalAutónoma de Mexico, Av. Universidad 3000 Edificio A, DelegaciónCoyoacán. Col. Cd Universitaria CP 04510 México.
[2]InstitutoNacional de Investigaciones Forestales, Agrícolas y Pecuarias, CENID-Salud Animal e Inocuidad, INIFAP, Carretera México-Toluca, Km 15.5. Col. Palo Alto, CP 05110. DelegaciónCuajimalpa, Ciudad de México.
[3]Instituto Mexicanodel Seguro Social, México.

Bovine mastitis is a disease with a high economic impact on dairy farms and it has been described that different species of Candida can cause it. The objectives of this study were to evidence the production of biofilm by strains of Candida *Kefyr*, to carry out a comparative evaluation of its production by tube and plate techniques, to determine its interaction with Escherichia coli and Streptococcus *dysgalactiae* isolated from bovine mastitis and know the drug susceptibility of each of them. The identification of C. *kefyr* was carried out by fermentation and assimilation of carbohydrates, the confirmation of the identification and minimum inhibitory concentration to *antimycotics* was carried out with *Vitek* 2 Systems. Plate and tube biofilm formation assays were performed in triplicate. Analysis of variance (ANOVA) and Tukey's test were performed, as well as Spearman's correlation to the data obtained. In plate tests, biofilm formation was demonstrated in seven C. *kefyr* isolates, as well as synergy in combinations with E. coli and S. *dysgalactia*e. However, the inoculum containing the three microorganisms behaved similarly to that containing only C. *kefyr* and S. *dysgalactiae*. It was possible to demonstrate a correlation between biofilm formation tests in tube and plate. This is the first report in Mexico of C. *kefyr,* as well as the production of biofilm in bovine clinical mastitis.

Key words: Mastitis, *Candida kefyr*, *Escherichia coli*, *Streptococcus dysgalactiae*, biofilm, drug susceptibility

INTRODUCTION

Mastitis is the inflammatory response of the mammary gland to a physical lesion or microbial infection. This condition has an enormous impact on dairy production, animal wellbeing and milk quality, and therefore on the economy of the production units (Ávila and Gutiérrez, 2018; Romero et al., 2018).

There have been more than 135 microorganisms identified as etiological agents of bovine mastitis, the

most frequent of which are *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus* spp and *Mycoplasma* spp (Watts, 1988). Other microorganisms such as yeast, mold, algae and virus can also cause an inflammatory process in the mammary gland (Watts, 1988*)*. Although mastitis incidence by fungi is generally low, they can be considered causal agents for mammary infections, when antimicrobial treatments are not effective (Bourtzi-Hatzopoulou et al., 2003; Krukowski et al., 2000).

Fungi are found mainly on soil; some yeast can be found as normal flora of mucous membranes such as in the vaginal, oral, rectal and ruminal cavities. They may get to colonize mammary gland skin in small amounts and generally, they are opportunistic microorganisms causing disease in conditions of immunosuppression (Hassan et al., 2018). The sources of origin are soil, plants, water, organic material, milker's hands, milk cups, and multi-dose syringes or treatments that may be contaminated, or which have not been handled hygienically which can cause the return of the condition, once treatment is suspended or improvement without any treatment. Transmission can happen through the milk cups, cow to cow, directly through the environment and from cow to cow during the milking process (Bourtzi-Hatzopoulou et al., 2003).

In general, yeast mastitis infections have a low presentation in commercial dairy farms, with a reported prevalence of around 3%. Nevertheless, there have been reports of higher prevalence such as in Brazil of up to 17.3% (De Casia dos Santos and Marin, 2005); in Greece, 6.2% (Bourtzi-Hatzopoulou et al., 2003) and in Poland, 7.07%, with *Candida krusey* isolated in 41.33% of cases and *C. kefyr* in 32% cases (Wawron et al., 2010). Other reports mention prevalence of 1 to 4% in cases of subclinical mastitis, and up to 25% in clinical mastitis, in dairy herds *(*Wawron et al., 2010; Bourtzi-Hatzopoulou et al., 2003).

During the last few decades, there has been an increase of infections caused by *Candida* (Sartori et al., 2014). When yeasts invade the mammary gland, they may cause a non-severe, generalized mastitis that is resistant to habitual treatments, and recur a few days after finishing treatment and that improve when there is no treatment. The most frequent microorganism that causes mastitis is *Candida albicans,* although other species have been described as mastitis causing, such as *Candida krusei*, *Candida parakrusei*, *Candida parapsilosis*, *Candida guillermondii*, *Candida famata*, *Candida utilis*, *Candida rugosa, Candida dirferi, Candida kefyr* (Costa et al., 1993; Krukowski et al., 2000; Segundo et al., 2011).

Many candida species produce hydrolytic enzymes, toxins and hemolysins, can adapt to different habitats and produce biofilm, which is a structured community imbedded in an extracellular matrix that is generally composed of water, carbohydrates, proteins and hexosamines *(*Panizo and Reviakina, 2001*)*, and adhered to a surface or to other microorganisms (Flemmins et al., 2016). It is one of the most important virulence factors since it ensures the permanence of bacteria in the mammary gland (Vanderhaeghen et al., 2014).

The ability to form biofilm is associated to infectious processes and to an increase in antifungal therapy resistance (Türkyilmaz and Kaynarca, 2010). Yeast may produce polysaccharides that make them more virulent, aggressive and difficult to eliminate (Olson et al., 2002; Panizo and Reviakina, 2001). In the case of *C. albicans* they produce biofilm in 40% of the cases, while other types of *Candida* produce biofilm in 60% of the cases (Panizo and Reviakina 2001).

Escherichia coli is one of the most frequent environmental microorganisms in bovine mastitis cases. Among its virulence factors are toxins, adhesins, and the production of capsule. Recurring *E. coli* mastitis may derive its persistence from the production of biofilm (Gomes et al., 2016). *Streptococcus dysgalactiae* is another frequent microorganism in bovine clinical and subclinical mastitis that produces virulence determinants such as fibronectin and fibrinogen (Gomes et al., 2016), as well as, produce biofilm, that makes eradication from dairy herds difficult (Olson et al., 2002). The objectives of this study were to compare the biofilm formation by the plate and tube techniques, to know the interaction in the film formation by *C. kefyr, E. coli* and *Streptococcus* as well as to know the antimicrobial susceptibility in each one of them.

MATERIALS AND METHODS

Samples

Microorganisms analyzed in this study were isolated from milk samples taken from a dairy farm in the State of Querétaro México and sent to CENID Animal Health and Food Safety of the INIFAP Institute. The milk samples came from cows with clinical mastitis, seeded into McConkey agar and Blood agar, and incubated at 37°C for 24 h. Observation of type and color of the colony was done, and a smear was Gram stained to verify purity of the culture. Once the presence of yeast was detected in nine samples (coded, A, B, C, D, E, F, G, H, I) they were seeded in Sabouraud Dextrose Agar, and incubated at 37°C for 24 to 48 h. In some samples, besides yeast isolates, Gram-positive and Gram-negative bacteria were found. These colonies were then purified, and identification of

*Corresponding author. E-mail: hernandezandrade@yahoo.com.
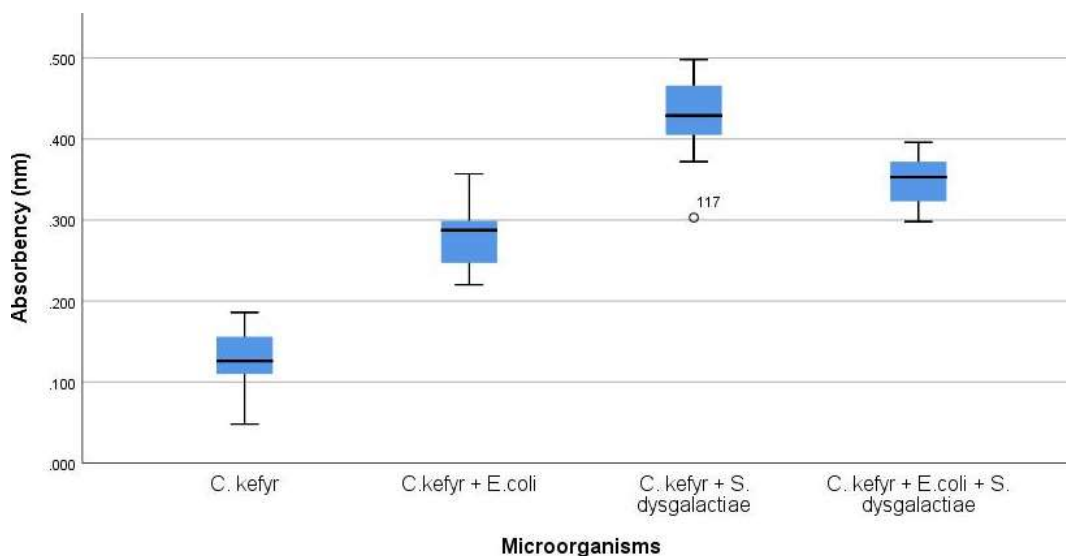
Figure 1. Comparison of absorbance obtained from groups of microorganisms in plate adherence tests. IBM SPSS Version 22

E. coli and *S. dysgalactiae* was made by Gram stain and traditional biochemical tests using identification tables.

Yeast identification biochemical tests

The methodology used was the one described by Castillo (1974).

Carbohydrate fermentation

A base broth with phenol red, with 1% concentrations added of maltose, lactose, dextrose or galactose was used.

Carbohydrate assimilation tests

Red phenol broth with carbohydrates added in the concentrations previously mentioned, plus 1% absolute ethylic alcohol was used. A positive test was considered to be that in which the media became turbid, evidencing the growth of yeast.

Automatized yeast identification

In parallel, yeast was identified with Vitek 2 Systems (bioMérieux) equipment using the Automatized Yeast Identification (YST) identification card, which contains 46 biochemical tests that determine use of carbon and nitrogen, as well as enzymatic activity.

Antifungal susceptibility with Vitek 2

The AST-YSO7 card was used to determine the minimum inhibitory concentration of *C. kefyr* strains such as Amphotericin (0.25 to 8 µg/ml), Caspofungin (0.25 to 4 µg/ml), Fluconazole (1 to 64 µg/ml), Flucytosine (1 to 64 µg/ml), Micafungin (0.06 to 4 µg/ml), Voriconazole (0.12 to 8 µg/ml).

Susceptibility to antibiotics test

Antimicrobial susceptibility of *E. coli* and *Streptococcus dysgalactiae* strains was obtained by the Kirby Bauer method (Leber et al., 2016).

Tube test biofilm

Tube tests were carried out separately for *C. kefyr*, *E. coli*, *S. dysgalactiae*, as well as for each of the combinations of *C. kefyr* and *E. coli*; *C. kefyr* and *Streptococcus dysgalactiae* (Figure 1).

Inoculum preparation

The technique proposed by Bonilla et al. (2012) was used, soytrypticase broth was inoculated with a fresh *Candida* culture, and incubated at 37°C for 48 h. Once incubated, the tubes were centrifuged at 1000 rpm for 10 min. Obtained precipitate was separated and placed in physiological saline solution in tubes for a second centrifugation for 10 min at 1000 rpm. The precipitate was again re-suspended in 3 ml of soy trypticase broth in polystyrene test tubes, incubated at 37°C for 48 h in a horizontal position. As a positive biofilm formation control, the *S. aureus* Cowan strain, commonly used as a positive control for mastitis research, was used separately and standardized with the McFarland 0.5 tube. The negative control tubes were one with sterile soy trypticase broth and another with a sterile saline solution. The tubes were washed three times with a pH 7.3, phosphate buffer solution (PBS), to remove microorganisms that were not adhered.

Staining and reading

After the wash they were set using absolute ethanol for 5 min, the excess was removed and then left to dry. Once dry, they were stained with a 0.1% crystal violet solution for one minute and rinsed with distilled water to remove excess stain. Positive tubes

were those in which the purple stained biofilm was observed; they were categorized as: (-) not detected, (+) sensitive (weak presence), (++) intermediate presence and (+++) strong presence.

Production of biofilm on plate

Inoculum preparation

Before washing and staining of the tubes, 100 µl of culture inoculated in the tubes was added to 100 µl of soy trypticase broth for the biofilm formation test. The assay was done in triplicate, using polystyrene microplates of 96 flat bottom wells for cell culture, and incubated for 24 h at 37°C aerobically. As positive control, the *S. aureus* Cowan strain was inoculated in soy trypticase broth at a dilution of 1:100, attained by placing 2 µl of inoculum in 198 µl of soy trypticase broth. After the incubation time, the broth was eliminated, and the wells of the microplate were washed three times with PBS; then fixed with absolute ethanol for 5 min. The excess ethanol was removed, and the plate left to dry for an hour.

Staining and reading

The plates were stained with a 0.1% crystal violet solution for 1 min, washed with distilled water to remove excess stain, and dried at room temperature. The plates were read using the ELISA Labsystemsmultiskan Ascent 354 microplate reader, at a 492 nm wavelength. Plate and tube tests were carried out in triplicate.

Statistical analysis

An average was obtained from the absorbency results of the three test repetitions and a analysis of variance test was applied (ANOVA); a means comparison test (Tukey) with a p≤0.05 significance was applied to verify if there was a statistical difference between the negative control and the isolates that were under analysis, as well as between the adherence of the following groups: *C. kefyr; C. kefyr + E. coli; C. kefyr + S. dysgalactiae;* and *C. kefyr + E. coli + S. dysgalactiae.* Spearman's bivariate correlation test was applied at 99% to determine if there was association between the adherence in tube and adherence on plate. The statistical analysis of the data was carried out using the statistical program IBM SPSS Statistics 22.0.

RESULTS

Identification

The nine yeast isolates (A, B, C, D, E, F, G, H, I) followed the same biochemical and carbohydrate assimilation pattern; therefore, they were classified as *C. kefyr* by biochemical methods and by Vitek 2. Two milk samples had *C. kefyr, S. dysgalactiae* and *E. coli*, while four milk samples had *C. kefyr* and *S. dysgalactiae*, and in the remaining, three, only *C. kefyr* was obtained*.*

Susceptibility to antimicrobial and antifungal drugs

S. dysgalactiae showed a similar pattern of susceptibility to the twelve antimicrobial drugs analyzed, of which it was susceptible only to cefalotin, cefotaxime and cefuroxime, which make evident the high degree of resistance that the isolated strains have (Table 1). *E. coli* were only sensitive to two antimicrobials of the twelve tested drugs: amikacin and trimethoprim/ sulfamethoxazole (Table 2). Minimum inhibitory concentrations for the *C. kefyr* strains were similar in the nine isolates: amphotericin, 0.25 µg/ml; caspofungin, 0.25 µg/ml; fluconazole, 1 µg/ml; flucytosine, 1 µg/ml; micafungin, 0.06 µg/ml; voriconazole, 0.12 µg/ml, and therefore they were classified as sensitive to these drugs.

Biofilm formation tube test

Tube tests were positive in all the cases; nevertheless, six isolates of *C. kefyr* presented the most evident adherence when compared to the other three isolates and negative controls. In the case of *E. coli* and *S. dysgalactiae*, these were also positive, although the positive control *S. aureus* always had a greater biofilm formation.

Formation of biofilm on plate

Growth of microorganisms was observed in all the wells, especially where the positive controls were placed. When subjected to staining there was some degree of biofilm formation in all the wells. There was a significant statistical difference (P≤0.05) between seven yeast strains, the *E. coli* and *S. dysgalactiae* strains*,* as well as the positive control, when compared with the negative control. Therefore, it can be said that the mentioned microorganisms had the capacity for forming biofilm on plate. Furthermore, the remaining yeasts did not have a significant difference with the negative control.

Regarding the interactions between *C. kefyr* and *E. coli, C. kefyr* and *S. dysgalactiae*, and *C. kefyr*, *E. coli* and *S. dysgalactiae,* they all had statistically significant differences with the negative control. Similarly, observed absorbances were statistically greater than those seen in samples that only had *C. kefyr* (Table 3).

Significant differences were found when comparing absorbances obtained from inoculum that contained *C. kefyr,* and the ones obtained from those that contained two or three microorganisms with the negative control, as well as when comparing mixed inoculums with those that only had *C. kefyr* (Table 4 and Figure 1).

In the tube tests, the results showed that all the isolates had some degree of biofilm formation. Most of the yeast samples, had a weak to intermediate film formation (+ to ++), while the samples that contained more than one microorganism had intermediate biofilm formation values (++); all the positive controls in all

Table 1. Results of susceptibility to antimicrobial drug tests performed on isolates of *S. dysgalactiae* associated to yeast.

Antimicrobial	A	B	C	D	E	F
Cefalotin	S	S	I	S	S	S
Ceftazidime	R	R	R	R	R	R
Erythromycin	I	I	R	R	R	R
Ampicillin	R	R	R	R	R	R
Tetracycline	R	R	R	R	R	R
Trimethoprim/Sulfamethoxazole	S	I	I	I	I	I
Cefotaxime	S	S	S	S	S	S
Gentamicin	R	R	R	R	R	R
Cefuroxime	S	S	S	S	S	S
Pefloxacin	I	R	R	R	R	R
Dicloxacillin	R	R	R	R	R	R
Penicillin	R	R	R	R	R	R

S=Sensitive, I= Intermediate, R=Resistant.
IBM SPSS Version 22

Table 2. Results of susceptibility to antimicrobial drug tests performed on isolates of *E. coli* associated to yeast.

Antimicrobial	A	B
Cefalotin	R	R
Chloramphenicol	R	R
Ceftriaxone	R	R
Ampicillin	R	R
Amikacin	S	S
Trimethoprim/Sulfamethoxazole	S	S
Cefotaxime	R	R
Gentamicin	R	R
Netilmicin	R	R
Pefloxacin	R	R
Carbenicillin	R	R
Nitrofurantoin	R	R

S=Sensitive, I= Intermediate, R=Resistant.
IBM SPSS Version 22

Table 3. Absorbency obtained in plate adherence tests.

Microorganism		A	B	C	D	E	F	G	H	I	(+) CTRL	(-) CTRL
C. kefyr	Mean	0.171	0.177*	0.079	0.132*	0.156*	0.249*	0.114	0.162*	0.164*	1.041*	0.059
	+/- SD	0.054	0.055	0.021	0.029	0.041	0.313	0.048	0.030	0.071	0.129	0.001
C. kefyr +	Mean	0.336*	0.318*	0.250*	0.317*	0.288*	0.307*	0.290*	0.289*	0.307*	1.207*	0.060
E. coli	+/- SD	0.035	0.061	0.030	0.031	0.040	0.071	0.058	0.020	0.030	0.103	0.003
C. kefyr +	Mean	0.443*	0.382*	0.437*	0.439*	0.410*	0.463*	0.447*	0.430*	0.450*	1.147*	0.060
S. dysgalactiae	+/- SD	0.025	0.040	0.045	0.037	0.020	0.029	0.047	0.018	0.033	0.141	0.001
C. kefyr +	Mean	0.267*	0.256*	0.228*	0.247*	0.244*	0.267*	0.271*	0.276*	0.245*	1.087*	0.055
E. coli + *S. dysgalactiae*	+/- SD	0.008	0.034	0.028	0.035	0.032	0.022	0.026	0.026	0.032	0.107	0.005

*Significant difference with the negative control (p≤0.05).
IBM SPSS Version 22

Table 4. Comparison of obtained absorbencies by microorganism groups, in plate adherence tests.

Microorganisms	Absorbances
Negative Control	0.063 +/- 0.06
C. kefyr	0.129 +/- 0.03 [A,C,D and E]
C. kefyr + E.coli	0.278 +/- 0.04 [A,B,D and E]
C. kefyr + S. dysgalactiae	0.432 +/- 0.03 [A,B and C]
C. kefyr + S. dysgalactiae + E. coli	0.349 +/- 0.02 [A,B and C]

Different letters show significant differences (p≤0.05)p
IBM SPSS Version 22

Table 5. Spearman's correlation testto determine the association between the tube and plate tests for biofilm formation.

			Absorbency	Tube
Spearman's Rho	Absorbency (p≤0.01)	Correlation Coefficient	1.000	0.689**
		Sig. (2-tailed)	.	0.002
		N	54	54
	Tube (p≤0.01)	Correlation Coefficient	0.689**	1.000
		Sig. (2-tailed)	0.002	.
		N	54	54

**Correlation test with confidence (p≤0.01).
IBM SPSS Version 22

repetitions showed strong adherence values (+++). When comparing the two methods for determining biofilm formation, Spearman's bivariate correlation test resulted in a Rho of 0.689 (Table 5).

DISCUSSION

The *C. kefyr* strains isolated from cases of bovine mastitis, demonstrated the capacity for biofilm formation on plastic surfaces; nevertheless, there is not much information available about biofilm formation by this microorganism when derived from animal origin strains. Most of the existing information has been obtained with human origin strains (Bonilla et al*.,* 2012; Hernandez et al., 2010*).*

Yet, the results found by Radford et al. (1998) in human origin strains, can be extrapolated in which certain variations may be evident in the formation of biofilm on different plastic material surfaces. The varying degrees of biofilm formation in the tube test, in which three yeast isolates had a lower degree of biofilm formation when compared with the rest, agree with the results of the biofilm production plate test, where these strains had no significant difference with the negative control. This can be associated with an irregular growth of the *C. kefyr* strains, which was more obvious in certain zones in tube as well as in plate, and in turn, may be associated to the seeding method or simply with the natural behavior of the yeast, as described by Vasilas and Molina (1992).

The fact that there was a significant difference in the formation of biofilm in the samples where the yeast was combined with a biofilm producing bacteria, could be an indication that, if these microorganisms are found together during an intramammary infection, it might make the ailment difficult to treat due to complications related to biofilm production by the microorganisms such as chronic infections and resistance to antimicrobials.

Interactions between biofilm producing microorganisms may be antagonistic, mutualistic or commensal (Chaves et al., 2007; Burmølle et al*.,* 2006*).* A synergy in the production of biofilm was found when the isolates of *C. kefyr, E. coli* and *S. dysgalactiae* were combined, since absorbance was increased significantly. Because the value or Rho reached 0.689 in Spearman correlation test, it can be inferred with high confidence (p≤0.01) that there is a good correlation between the biofilm formation tests carried out in tube and those carried out in plate.

It seems the increase in antibiotics use during the last

few years, has contributed to an increase of mycotic mastitis incidence. It has been suggested that yeast or fungi multiply during antibiotic therapy, stimulated by the elimination of antagonistic bacterial flora and a reduction of vitamin A in the mammary gland tissue (Wawron et al., 2010; Costa et al., 1993*).* Mycotic mastitis are considered environmental mastitis, predominantly caused by yeast of the *Candida* genus (Watts*,* 1988*),*

Türkyilmaz and Kaynarca (2010), isolated from a total of 339 milk samples, 12.1% yeasts, of these, 36.6% were *C. krusei*, 7.3% *C. kefyr*, and 4.9% *C. guillermondii*. Furthermore, of the latter they reported that in 36.6% of them there was the formation of biofilm, as well as in 7 strains of *C. krusei* and in 4 of *Candida kefyr.*

Vinitha and Ballal (2007) reported that the production of biofilm is less frequent in *Candida albicans* (42.9%) while other species of *Candida* produce more, up to 63.4%. Studies carried out in Lublin, Poland, isolated yeasts in 9.6% of 604 milk samples, of which the most predominant was *C. kefyr* with 24.1% (Krukowski et al.*,* 2000).

In samples from mammary glands with subclinical mastitis, 9.92% of the 383 samples had *Candida,* identifying 15 different species. The most frequent isolate was *C. albicans* 13.15% (5/38) and *C. krusei* (7.89%; 3/38). In 379 milk samples of clinical mastitis cases, the most frequent isolate was *C. krusei* (40.24%), while other *Candida* species, *C. kefyr*a mongst them, had a frequency of 1.7% (Ksouri et al.*,* 2015).

According to studies by Wawron et al. (2010) the increase in the use of antibiotics in the past few years is a key factor that contributes to the higher incidence of yeast in the mammary gland as they demonstrated that *Candida* spp*.* was the most frequently isolated etiological agent in mycotic mastitis in cows, while the most abundant species included *C. krusei* and *C. kefyr*. There is great difference in the reports around the world of yeast isolations in cases of bovine mastitis (Türkyilmaz and Kaynarca *,* 2010).

Milanov et al. (2014) studied sensitivity against antifungal drugs and found that two isolates of *C. kefyr* showed resistance against nystatin, which is commonly used for treating yeast mastitis. Other isolates have shown resistance to fluconazole and Flucytosine, which are commonly used in the treatment of human infections (Hassan et al., 2018; Ochiuzzi et al., 2014). In contrast, in this study isolated yeasts were sensitive to the anti-mycotic drugs tested.

Conclusion

The relevance of the capacity of *C. kefyr* strains to form biofilm in this study lies on the secondary implications, since their presence may include the adsorption of other microorganisms and thus establish protection when confronted with antimicrobial and/or antifungal agents. This effect was verified in this study, with the presence of *S. dysgalactiae* and *E. coli* associated to *C. kefyr,* in cases of clinical mastitis.

We conclude that *C. kefyr* is capable of forming biofilm on plastic materials, although not as strong as that which is generated by other microorganisms such as some *S. aureus* strains, which can then be converted into a source of infection if it adheres to the materials from which the mechanical milking equipment are made of. This situation may generate cases of mastitis where the presence of these yeasts is complicated with the presence of other microorganisms such as *S. dysgalactiae* or *E. coli*, amongst others.

A good association was found between the plate and tube tests for biofilm formation, and therefore the latter could be used as an indicator of biofilm formation by *C. kefyr* on plastic surfaces.

Likewise, an appropriate handling of mammary gland infectious processes is important, since the finding of microorganisms highly resistant to most antimicrobial agents used routinely makes evident the indiscriminate use of those drugs, and therefore it is recommendable to use susceptibility tests in all the mastitis control programs in dairy farms.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENT

REFERENCES

Ávila TS, Gutiérrez Ch AJ (2018). Producción de leche con ganadobovino. 3aedición.México AMMVEB, A.C. ISBN 9786074480153.

Bonilla RY, Moreno MV, Muñoz HB, Palma CM (2012). Adherencia *in vitro* de *Candida albicans* en tresdiferentesacondicionadores de tejidosusados en prostodoncia total. RevistaOdontológica Mexicana 16(1):40-45.

Bourtzi-Hatzopoulou E, Zdragas A, Petriduo E, Filiosis GT (2003). Yeast as a causative agent of bovine mastitis in Greece. Journal of the Hellenic Veterinary Medical Society 54(2):105-110.

Burmølle M, Webb JS, Rao D, Hansen LH, Sorensen JS, Kjelleberg S (2006). Enhanced biofilm formation and increased resistance to antimicrobial agents and bacterial invasion are caused by synergistic multispecies biofilms. Applied and Environmental Microbiology 72(6):396-3923.

Castillo TE (1974). Algunosaspectosbioquímicos indispensables para la identificación de especies de *Candida.* Revistacolombiana de Cienciasquímico-Farmacéuticas 3(1):41-66.

Chaves SL, Somoes M, Joao VM (2007). Biofilm interactions between distinct bacterial genera isolated from drinking water. Applied and Environmental Microbiology 73(19):6192-6200.

Costa EO, Gandra CR, Pires MF, Coutinho SD. Castilho W, Teixeira M (1993). Survey of bovine mycotic mastitis in dairy herds in the State. Mycopathologia 124(1):13-17.

De Casia dos Santos R, Marin JM (2005). Isolation of *Candida*spp from mastitic bovine milk in Brazil.Mycopathologia 159(2):251-253.

Flemming HC, Wingender J, Szewzyk U, Steinberg P, Rice SA, Kjelleberg S (2016). Biofilms: an emergent form of bacterial life. Nature Reviews: Microbiology 14(9):563-575.

Gomes F, Saavedra MJ, Henriques M (2016). Bovine mastitis disease/pathogenicity: evidence of the potential role of microbial biofilms. Pathogens and Disease 74(3):ftw006

Hassan Y, Alhassan AS, Tian Lung TL (2018). *Candida albicans* interdigital foot infection: A case report highlighting the importance of antifungal susceptibility testing. African Journal of Microbiology Research 12(36):889-896.

Hernandez SSE, Gonzalez RAS, Rueda-Gordillo F (2010). Capacidad de adhesión de cepas de *Candida albicans*aisladas de unapoblación de niñosportadoressanos. Revistaodontológica Latinoamericana 2(2):33-37.

Krukowski H, Tietze M, Majewski T, Rózanski P (2000). Survey of yeast mastitis in dairy herds of small-type farms in the Lubrin region, Poland. Mycopathologia 150(1):5-7.

Ksouri S, Djebir S, Hadef Y, Benakhla A (2015). Survey of bovine mycotic mastitis in different mammary gland statuses in two north-eastern regions of Algeria.Mycopathologia 179(3-4):327-31.

Leber A (2016). Clinical Microbiology Procedures Handbook 4th (2016) edition.ASM Press.

Milanov D, Prunic B, Velhner M, Bojkovski J (2014). Diagnosis of yeast mastitis in dairy cows. LucrariStiintificemedicinaVeterinara 47(1) Timisoara.

Ochiuzzi ME, Arechavala A, Guelfand L, Maldonado I, Soloaga R y Red de Micología CABA, Argentina (2014). Evaluación de lastarjetas AST-YSO1 del sistemaVitek 2 paradeterminar la sensibilidad a antifúngicos de levadurasdelgenero*Candida*. Revista Argentina de Microbiología 46(2):111-118.

Olson ME, Ceri H, Morck DW, Buret GA, Read RP (2002). Biofilm bacteria: formation and comparative susceptibility to antibiotics. Canadian Journal Veterinary Research 66(2):86-92.

Panizo MM, Reviakina V (2001). Adhesinas y receptoresinvolucrados en el fenómeno de adherencia de *Candida albicans* a lascélulasepiteliales. Revista de laSociedadVenezolana deMicrobiología 21(1):05-11.

Radford DR, Sweet SP, Challacombe SJ, Walter JD (1998). Adherence of *Candidaalbicans* to denture-base materials with different surface finishes. Journal of Dentistry 26(7):577-583.

Romero J, Benavides E, Meza C (2018). Assessing financial impacts of subclinical mastitis on Colombian Dairy Farms. Frontiers in Veterinary Science. https://doi.org/10.3389/fvets.2018.00273

Sartori LCA, Santos RC, Marin JM (2014). Identification of *Candida* species isolated from cows suffering mastitis in four Brazilian states. Arquivo Brasileiro deMedicinaVeterinária eZootecnia 6(5):1615-1617.

Segundo ZC, Cervantes ORA, Ducoing WAE, De la Peña MA, Villa TL (2011). Yeasts isolation from bovine mammary glands under different mastitis status in the Mexican High Plateau. RevistaIberoamericana de Micología. 28(2):79-82.

Türkyilmaz S, Kaynarca S (2010). The slime production by yeasts isolated from subclinical mastitic cows. Acta Veterinary Brno 79(4):581-586, doi: 10.2754/avb201079040581

Vanderhaeghen W, Piepers S, Leroy F, Van Coillie E, Haesebrouck F, De Vliegher S (2014). Invited review: Effect, persistence and virulence of coagulase-negative *Staphylococcus* species associated with ruminant udder health. Journal of Dairy Science 97(9):5275-5293.

Vasilas A, Molina L (1992). The influence of morphological variation on *Candida albicans* adhesion to denture acrylic *in vitro*. Archives of Oral Biology 37(8):613-622.

Vinitha M, Ballal M (2007): Biofilm as virulence marker in *Candida* isolated from blood. World Journal of Medical Sciences 2(1):46-48.

Watts JL (1988): Etiological agents of bovine mastitis. Veterinary Microbiology 16(1):41-66.

Wawron W, Bochniarz R, Piech T (2010). Yeast mastitis in dairy cows in the middle-eastern part of Poland. Bulletin of the Veterinary Institute in Pulawy 54(2):201-204.