XII CONGRESO ARGENTINO DE MICROBIOLOGIA GENERAL

2 al 4 de agosto de 2017

San Miguel de Tucumán | ARGENTINA

SAMIGE

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MM-026

ANTIBIOFILM COMBINATION OF USNIC ACID WITH FLUCONAZOLE ON RESISTANT*Candida albicans*

Mariana Peralta[1 2], José L Cabrera[1 2], María G Paraje[1 3]

[1]CONICET, Instituto Multidisciplinario de Biología Vegetal (IMBIV). [2]Fac. de Cs Químicas, Universidad Nacional de Córdoba. [3]Cát. de Microbiología, Fac. de Cs Exactas Físicas y Naturales, Universidad Nacional de Córdoba.

gabrielaparaje@gmail.com

Treatment of *Candida* infections is often difficult due to, between others factors, the ability of *Candida* species to form biofilms. These highly resistant structures exhibit resistance to a variety of antifungal agents with clinical use. Therefore, combining them with compounds obtained from natural sources seems to be one of the strategies in order to restore the sensitivity of the microorganism to conventional antifungals such as azole drugs. In previous works, we reported inhibitory activity of usnic acid (UA), a natural compound obtained from lichens, against azole-resistant *Candida albicans* biofilm. The biofilms inhibitory concentration (BIC) was 4 mg/ml compared to fluconazole (FLZ, BIC, 2 mg/ml) with inhibitions percentages of about 70%. The present study investigated the sensitization (restoring the sensitivity of the microorganism to azole drugs used in the clinic) of azole-resistant *C. albicans* biofilms to FLZ by combining it with an active compound (UA) obtained from Argentinean native flora (*Usnea amblyclada*). UA was purified from the benzene extract of lichen *U. amblyoclada*. An azole-resistant strain of *C. albicans* isolated from the oral cavity (RCa) that overexpresses efflux transporters genes of type CDR1, CDR2 and MDR1 was used. Biofilm formation was measured by adhesion to a 96-well plate and quantified by Crystal Violet (CV) staining and spectrophotometric reading of Optical Density (OD) at 595 nm. The biofilm biomass unit (BBU) was defined as 0.1DO595nm = 1UBB. For antifungal activity determination different concentrations of UA (1 to 4 mg/ml) dissolved in dimethyl sulfoxide (DMSO), FLZ (0.5 to 2 mg/ml) or their combinations were added to each well containing the mature biofilm and incubated at 37 °C for 48h. The counts of Colony Forming Units (CFU/ml) were performed for BBU correlation studies. For Scanning Confocal Laser Microscopy (SCLM), the samples were stained with Calcofluor White (0.05% v/v). UA and FLZ combined at concentrations four-fold lower than their BICs had a greater inhibitory effect on biofilms. In fact, the combination of UA (1 mg/ml) and FLZ (0.5 mg/ml) achieved an inhibition of 79, 82% while UA and FLZ combined at 1 mg/ml and 0.5 mg/ml respectively, almost eradicated de mature biofilm with an inhibition of 97.69% (*$p < 0.01$). Analysis by SCLM showed a considerable decrease in biomass of biofilms treated with the combination of UA (1 mg/ml) and FLZ (0.5 mg/ml), compared to untreated RCa biofilms (* $p < 0.01$). These results suggest that the combination of UA with FLZ effect enhanced the activity of FLZ in the treatment of azole-resistant of *C. albicans* biofilms. This promising action would imply an improvement of the therapeutics due to the decrease of the concentrations used of antifungal drugs.

MM-027

PFGE PATTERNS OF *Escherichia coli* ISOLATED FROM DAIRY CATTLE PRODUCTION ENVIRONMENT IN BRAZIL

Greicine França Bronzato[2], María Florencia Cerioli[1], Shana Mattos de Oliveira Coelho[2], Mirta Lasagno[1], Elina Reinoso[1]

[1]Universidad Nacional de Río Cuarto. [2]Universidade Federal Rural do Rio do Janeiro.

florenciacerioli@hotmail.com

Escherichia coli has been described as prevalent and highly pathogenic in environmental mastitis etiology. Molecular typing is a powerful tool that can provide information about genetic characteristics of microorganism responsible for this disease. The objective of this work was to evaluate the genetic diversity of *E. coli* present in the dairy cattle production environment through the molecular typing technique *Pulsed Field Gel Electrophoresis* (PFGE). Additionally, circulating clones causing bovine mastitis present in the milk and feces of the bovine as well as in the water used in the management of these animals was determined. 282 milk samples were collected from 94 lactating cows in three consecutive weeks in summer, winter, spring and autumn. We also collected 94 samples of fecal material from these animals and water samples from nineteen different points related to the milk production line. Thus, 152 strains of *E. coli* were obtained through the phenotypic analysis. These strains were investigated by virulence genes such as Intimin (*eae*A), Shiga toxin (*stx*I and *stx*II), thermolabile (*LT*) and thermostable (*ST*) enterotoxins, invasiveness (*ial*) and enteroaggregative *E. coli* (*eagg*). Furthermore, genes associated with adherence were analyzed as fimbria F1 (*fim*H), fimbria curli (*csg*A) and antigen 43 (*flu*). According to the genes evaluated, the *fimh*, *csgA* and *flu* genes were prevalent in strains isolated within the dairy cattle production environment, being *fimh* the most detected gene in the 72.2% of the strains. The *flu* gene was not detected in water samples. On the other hand, percentage of genes related to the production of toxins *eaeA*, LT and *stxI* were detected in the 11.1% of the strains. It was not possible to detect other genes related to the production of toxins *stxII*, ST, *ial*, *eagg* in the strains tested. Profiles based on the presence virulence genes were determined to select 18 strains, which were assayed by PFGE. Among the 18 isolates, 16 different PFGE patterns were observed. Two clusters with 100% of homology, grouping 2 strains each other were found. One of them clustered two strains isolated from milk that share the same virulence genes. The present study describes *E coli* genotypes associated with the milk production environment and shows that there is no predominant PFGE pattern and no association between virulence genes analyzed and PFGE patterns was found.