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Original Research Article

Antifungal effect of cow's urine distillate on *Candida* species

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ABSTRACT

Background: Increase in resistance of *Candida* species, to routinely used antifungal agents has necessitated the quest for new drugs. Few studies have revealed that cow's urine can suppress the growth of pathogenic fungi. However there is no published report on antifungal effects of cow's urine on clinical *Candida* isolates.

Objective: The present study aims at exploring the antifungal potential of cow's urine on clinical isolates of *Candida* species.

Material & methods: In this *in-vitro* experimental study four standard strains and 37 clinical isolates of *Candida* species were tested for their susceptibility to amphotericin B, fluconazole and voriconazole, by disk diffusion method. Detection of MIC of cow's urine for the *Candida* isolates was done by agar dilution method using 20–50% concentration of cow's urine.

Results: Clinical isolates of *Candida albicans* $n = 22$ (59.5%) *Candida glabrata* $n = 6$ (16.2%), *Candida tropicalis* $n = 3$ (8.1%) and other *Candida* species $n = 6$ were tested for their antifungal susceptibility. Among them, 18.9% were resistant to voriconazole, 24.3% to amphotericin B and 35.1% to fluconazole. Statistically significant association was observed between susceptibility of voriconazole and that of cow's urine ($p = 0.045$). *C. albicans* ATCC14053, *Candida parapsilosis* ATCC22019 and 75.7% of clinical isolates of *Candida* were susceptible to cow's urine.

Conclusions: Cow's urine distillate has concentration dependent inhibitory effect on *Candida* species and is effective on the isolates that are either resistant or sensitive to the routinely used antifungal agents.

1. Introduction

Continuous emergence of antifungal resistance across the globe has necessitated a quest for new antifungal agents. According to Indian ancient Ayurvedic texts such as “Charak Samhita”, “Ashtanga Sangraha” and “Atharva Veda”, cow's urine has an indelible place in Ayurveda and has been believed to be one of the animal secretions possessing the most therapeutic significances since the ancient time. Cow urine therapy has been practiced by a large number of people for the treatment of various diseases using Panchagavya or Panchakavyam which is made of cow dung, milk, ghee, curd, and urine [1].

Cow Urine Therapy and Research Institute, Indore has shown success in curing ten patients with long term fungal infection of throat which resisted to ordinary treatment [2]. It has also been used in the treatment of other diseases like hypertension, cancer, diabetes mellitus, ophthalmic disorders, urological syndrome, gynecological disorders, skin diseases, etc. Few studies have also shown the ability of cow's urine in suppressing the growth of pathogenic microorganisms like fungi, bacteria and even helminths [1,3–10]. However a thorough scientific validation is required to establish the efficacy of cow's urine for its worldwide acceptance as an alternative to antifungal agent.

Candidiasis is an opportunistic fungal infection caused by different *Candida* species like *Candida albicans*, *Candida glabrata*, *Candida parapsilosis*, *Candida tropicalis*, and *Candida krusei* which is a normal inhabitant in humans. Invasive candidiasis is a major health care issue in people who are on long term broad spectrum antibiotics treatment, malignancy, immunocompromised state and other predisposing factors [11]. Recent statistics revealed that *Candida* species showed a decrease in susceptibility to azoles such as fluconazole, the most common drug used in the treatment of candidiasis and also to the novel antifungal agent like

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echinocandins. The other alternative such as amphotericin B which are easily available in most of the places but are associated with more adverse effects in comparison with azoles and echinocandins, has now become futile in the treatment of candidiasis caused by some of the *Candida* species, such as *Candida lusitaniae* [12]. This emerging antifungal resistance could lead to consequences like administration of more expensive alternative antifungals, prolonged hospitalization, and rise in morbidity and mortality among those high risk individuals.

Hence we aimed at exploring the antifungal potential of cow's urine distillate on *Candida* species isolated from clinical samples which may help to suggest an alternative and cost effective treatment for drug resistant Candidal infections.

2. Materials and methods

This was an *in-vitro* experimental study carried out for a period of four months (01 April 2015 to 30 July 2015) in which distilled cow's urine was tested for its antifungal effect on standard strains and clinical isolates of *Candida* species after obtaining ethical clearance form Institutional Ethics Committee.

2.1. Cow's urine

Distilled cow's urine (known as Arka in Sanskrit) of a special breed (Kapila) of disease free cow, confined mainly to South Karnataka, was obtained from local cow yard at Surabhivana, Kompadavu, Mangalore.

2.2. Strains used in the study

Standard strains like *C. albicans* ATCC 14053 and *C. tropicalis* ATCC 66029 from HiMedia Laboratories Pvt Ltd., *C. parapsilosis* ATCC 22019 and *C. krusei* ATCC 6258 from bioMérieux Pvt Ltd. were used to test the anti-fungal effect of cow's urine on these strains and to standardize the amount of urine required for testing clinical Candida isolates. Thirty *Candida* species, isolated from different clinical samples were included in the study by following convenient non-random sampling method with 95% confidence level and 90% power with reference to a study conducted by Sathasivam et al. (2010) [1]. Sample size was found to be 30 when calculated using the formula: $n = (Za^2\sigma^2)/d^2$. All the Candida isolates used in the study were identified by standard biochemical reaction [13] and maintained at 4 °C on Sabouraud's Dextrose agar slope.

2.3. Sterility check of cow's urine distillate

Before testing for antifungal effect, sterility check of cow's urine distillate was done by inoculating 1 ml urine distillate into 9 ml of Brain Heart Infusion (BHI) broth. BHI broth was incubated at 37 °C for 4 weeks. At the intervals of 48 h, 4th day 7th day, 14th day, 21st day & 30th day, subcultures were done from the incubated BHI broth onto blood agar and MacConkey's agar and Sabouraud's dextrose agar plates. Inoculated plates were incubated at 37 °C for 48 h and checked for any bacterial or fungal growth [13].

2.4. Detection of MIC of cows urine for standard and clinical strains of Candida species

Minimum inhibitory concentration (MIC) of sterile cow's urine preparations on clinically isolated *Candida* species and standard strains of *Candida* species were determined by agar dilution method. Mueller Hinton agar (MHA) containing 0.5 μg/ml methylene blue, 2% glucose and different concentrations of cow's urine distillate (CUD) ranging from 20 to 50% were prepared. Ten microliters of standard strains of *Candida* species as well as clinical isolates grown in Mueller Hinton broth for 24 h, whose turbidity was adjusted to 0.5 Mac Farland standard (10^6 CFU/ml) was inoculated onto MHA containing different concentration of CUD as well as MHA without CUD. All plates were incubated at 27 °C for 24 h. The highest dilution of the cow's urine that did not show visible growth was taken as MIC. MHA without cow's urine distillate acted as growth control [4,14].

2.5. Susceptibility testing for routinely used antifungal drugs

Anti-fungal susceptibility to routinely used drugs like amphotericin B (100 units), fluconazole (25 μg) and voriconazole (1 μg), was done by disk diffusion method, using Muller Hinton agar supplemented with methylene blue. Results were interpreted as per CLSI guidelines [15,16].

3. Results

3.1. Sterility of cow's urine

Distilled Cow's urine tested were found to be sterile even after 30 days of incubation in BHI broth. These sterility checked urine was used in the study to check their antifungal effect.

3.2. Candida species isolated from clinical specimens

A total of 37 *Candida* strains isolated from clinical samples like urine (56.8%), blood (13.5%), sputum (10.8%) and other specimens (18.9%) like ascetic fluid, high vaginal swab, pus, suction tip, central line, endotracheal tube and maxillary sinus were included in the study to know the antifungal effect of cow's urine distillate. These isolates consisted of *C. albicans* (59.5%) followed by *C. glabrata* (16.2%), *C. tropicalis*, *C. krusei*, *C. parapsilosis* and other Candida species like *Candida haemulonii*. Agewise and sexwise distribution of *Candida* species among different clinical samples are shown in Tables 1 and 2.

3.3. Effect of cow's urine on standard strains and clinical isolates of Candida species

The standard strains of *C. albicans* ATCC 14053, *C. parapsilosis* ATCC 22019, *C. tropicalis* ATCC 66029 and *C. krusei* ATCC 6258 were tested with the routinely used antifungal drugs and cow's urine and the results are shown in Table 3.

Susceptibility to routinely used antifungal agents was found to be interesting. 18.9% of the clinical isolates were resistant to voriconazole (1 μg), 24.3% of the isolates to amphotericin B (100 units) and 35.1% of the isolates to fluconazole (25 μg) (Table 4). However, 24.3% of clinical Candida isolates were not inhibited by cow's urine and these included *C. albicans* ($n = 6$), *C. glabrata* ($n = 2$) and *C. krusei* ($n = 1$) (Table 5).

Among the voriconazole resistant clinical isolates, 4 (57.1%) were found to be resistant to cow's urine, whereas 42.9% of isolates which were resistant to voriconazole were sensitive to cow's urine. However, most of the isolates resistant to amphotericin B (5 out of 9 or 55.6%) and fluconazole (8 out of 13 or 61.5%) were found to be susceptible to cow's urine. Moreover, cow's urine was also found to be effective against those clinical isolates that were sensitive to the routinely used antifungal agents. By using Fisher's Exact Test, there was a statistically significant association between susceptibility to voriconazole and that of cow's urine (p value = 0.045, p value less than 0.05 is significant) (Table 6).

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