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Antimicrobial Effects of Commonly Using Antifungal Vaginal Creams Against Vaginal Microflora [Version 1, 1 Approved]

Epiphania Nghinambwedi Haimbodi, Munyaradzi Mukesi* and Sylvester Rodgers Moyo

Department of Health Sciences, Faculty of Health and Applied Sciences, Namibia University of Science and Technology, Namibia

***Corresponding author:** Munyaradzi Mukesi, Department of Health Sciences, Faculty of Health and Applied Sciences, Namibia University of Science and Technology, Namibia, Email: mmukesi@nust.na

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Abstract

Over a billion of women worldwide suffer from non-sexually transmitted urogenital infections such as bacterial vaginosis, yeast vaginitis and urinary tract infections. These infections can be treated with antifungal and antibacterial agents. The use of fungicidal vaginal creams disturbs the vaginal environment therefore pre-disposing women to other infections. The study was conducted to assess the in vitro antibacterial activities of five antifungal vaginal creams commonly used in Namibia against the vaginal microflora. The organisms selected as vaginal flora were *Staphylococcus aureus*, *Staphylococcus epidermidis*, Lactobacillus species, *Escherichia coli*, *Streptococcus pyogenes*, *Streptococcus agalactiae*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. Data was analysed using Microsoft excel and results were presented in tables. The results obtained revealed that antifungal vaginal creams such as GZ, CS, MS and NS, with the exception of CZ contain antibacterial agents which exhibited antibacterial activity against *Staphylococcus aureus* and *Staphylococcus epidermidis*. The antimicrobial activity of antifungal vaginal creams on staphylococcus strains can be due to individual ingredients such as clotrimazole or a combination of ingredients such as clotrimazole, imidurea and nipastat in the creams. Presence of these antibacterial agents in the creams can cause disturbance of the vaginal flora.

Introduction

Vaginal yeast infection is defined by the Centres for Disease Control and Prevention (CDC) guideline as an infection of the vagina that causes itching and burning of the vulva, the area around the vagina [1]. About three out of four women get yeast infections at some points in their life. A small amount of yeast is naturally found in the vagina of many women along with certain harmless bacteria [1]. However, when yeast grows excessively, an infection may occur. Some women's immune system fights off the infection; others cannot and hence require medication. Approximately 15% of women have recurring yeast infections. Yeast grows in the vagina because it prefers a warm, dark and moist environment rich in sugar and biological nutrients [2].

An infection occurs only if yeast grows profusely. Yeast may grow excessively if the vagina's acidity or bacterial environments get out of balance. These imbalances may be caused by various factors such as using antimicrobial medications or using the birth control pills or due to a lowered immune system, diabetes, pregnancy, obesity or illness [2]. Yeast infections are however treated by suppositories, ointments or creams. Oral medications may however sometimes be administered to act on the gastrointestinal system where yeast may also grow and cause re-contamination of the vagina. For ointments and creams, the medication is inserted into the vagina [1].

The use of fungicidal vaginal creams possessing antibacterial effects against normal flora of the vagina can lead to disturbance of the vaginal environment. The vaginal flora is of utmost importance in maintaining the vaginal environment, by producing lactic acid and a variety of antimicrobial compounds [3]. The use of these antifungal vaginal creams predisposes women to vaginal infections. This necessitates the need to conduct studies of this nature, to assess the effects of topical fungicidal creams on the microflora of the vagina and determine which antifungal cream exhibit antimicrobial effects against the vaginal flora.

Infection of the Lower Female Genital Tract

Gynaecological infections are among women's common diseases [4]. These infections are classified into three groups which include, sexually transmitted diseases (STDs), such as chlamydia, gonorrhoea and Human immunodeficiency virus (HIV); endogenous infections, which are caused by overgrowth of organisms normally present in the genital tract of healthy women, such as bacterial vaginosis or vulvovaginal candidiasis; and iatrogenic infections, which are associated with improperly performed medical procedures such as unsafe abortion or poor delivery practices [5].

These infections have become a burden for women as they are often asymptomatic or the symptoms are not recognizable [6]. Vaginitis is the most prevalent gynaecological problem causing women to seek for treatment and is responsible for 10 million physician visits annually [4]. Although these infections are rarely life-threatening, morbidity related to these often deprive society from important contributions made by women in terms of economic, social and cultural development [7].

In 2006 the prevalence of vaginitis in American women's clinics was reported to be 5 – 15% and 32 – 64% for sexually transmitted diseases [4]. Recently, the prevalence of vaginal candidiasis has increased noticeably, making it the second highest prevalent vaginal infection worldwide [4]. Approximately 70% of all women experience a mycotic vulvovaginitis at least once during their life time. In addition, roughly 40-50% of women may experience a secondary episode of candidiasis vulvovaginitis in their life whereas 5% of women report the recurrence of candidiasis vulvovaginitis [8]

Annually, 13 million cases of mycotic vulvovaginitis occur in the United States (US) [9]. In one study, prevalence of candida vaginitis was reported in patients without vaginitis history as 58.33% and in patients with recurred vaginitis as 33.3% [10]. A similar study also reported presence of candidiasis vulvovaginitis in 20.47% of women in the US [8].

Africa is no exception when it comes to vaginitis as a result of candida. The prevalence of candida vaginitis among patients in Ibadan, Southwestern Nigeria was reported to be 60% of which 20% presented with vaginitis [11]. This indicates extremely high prevalence of candida in Nigeria. A similar study reported candida prevalence among vaginitis symptomatic pregnant women aged 26 – 30 years attending a state university antenatal clinic in Awka Nigeria to be 63.6% [12]. This shows that pregnant women are at greater risk of vaginitis hence require routine medical examination and appropriate treatment when infected.

Antifungal Creams

Antifungal creams are used to cure a variety of conditions including jock itch, athlete's foot, yeast infections as well as finger and toenail infections. They usually consist of a combination of pharmaceuticals or herbs, nutrients and other natural sources that have antifungal properties [13]. Oral antifungal creams are taken to eradicate yeast in the gut and systemic candidiasis, while topical are used on particular areas of the body, such as feet or vagina [13].

The ingredients found on topical creams may include miconazole, econazole, tioconazole and clotrimazole [4]. Sometimes the creams include hydrocortisone, which reduces the itching that often accompanies infections related to fungal growth. Antifungals work by exploiting differences between mammalian and fungal cells to kill the fungal organism with fewer adverse effects to the host [13]. Unlike bacteria, both fungi and humans are eukaryotes, thus, their cells are similar at biological level. This makes it more difficult to discover drugs that target fungi without affecting human cells.

Many of the antifungal drugs cause side-effects such as increase of hepatic enzymes, painful urination and depression, which may occur as a result of systematic drug absorption [4]. Side effects such as irritation or contact dermatitis, neutropenia and mental depression may also be observed. Systemic administration of these drugs during pregnancy causes embryo toxicity. Therefore, antifungal drugs should be administered with caution during pregnancy and even breast-feeding, since it is not clear if they enter into mother's breast milk. These drugs can be life-threatening if they are not used properly [13].

The different types of antifungal vaginal creams contain clotrimazole, preserved with Benzyl alcohol or preserved with of Nipastat and Imidurea. Others contain 10mg of clotrimazole and of Nipastat, and Biopure 100 as preservatives.

Human Normal Flora

Different microbial flora is associated with the skin and mucous membranes of every human being from shortly after birth until death [14]. This bacterial population constitutes the normal microbial flora. The normal microbial flora is relatively stable, with specific genera populating various body regions during particular periods in an individual's life [15]. Microorganisms of the normal flora may aid the host, harm the host or exist as commensals. Even though most elements of the normal microbial flora inhabiting the human skin, nails, eyes, oropharynx, genitalia and gastrointestinal tract are harmless in healthy individuals, these organisms frequently cause disease in immuno-compromised individuals [15]. The normal flora in humans usually develops in an organized sequence after birth [14]. The main factor determining the composition of the normal flora in the body region is the nature of the indigenous environment, which is determined by pH, temperature, redox potential as well as oxygen, water and nutrient levels. The factors such as peristalsis, saliva, lysozyme secretion and secretion of immunoglobulins also play roles in flora control [14]

Normal microflora acts as a barrier against colonization of the vagina by pathogenic microorganisms and against overgrowth of already present opportunistic microorganisms [16]. The use of antimicrobial agents to slow down or kill pathogenic microbes often kills beneficial bacteria causing lethal health effects. Antimicrobial agents are inclined to alter the flora, allowing pathogenic microbes to overgrow and cause diseases [17].

Several factors influence the extent to which antimicrobial agents will destroy the normal microflora. Chief among these factors is the complete absorption of orally administered drugs [18]. Poorly absorbed agents can reach the intestine in an active form where they destroy susceptible microorganisms and change the ecologic balance [16].

Studies done on the effects of antimicrobial agents on normal microbial flora indicated that the suppression of the intestinal microflora by antimicrobial agents creates a microbiologic vacuum readily filled by exogenous pathogens or by overgrowth of commensal microorganisms [18].

Vaginal Flora

Vaginal flora is composed of bacteria that live inside the vagina. The type of bacterial flora found in the vagina depends on the age, pH and hormonal levels of the host. Lactobacillus species predominate in female infants during the first month of life [19]. Glycogen secretion seems to cease from about 1 month of age to puberty. During this time, diphtheroids, *Staphylococcus epidermidis*, and *Escherichia coli* predominate at pH 7. At puberty, glycogen secretion resumes, the pH drops and women acquire an adult flora in which *Lactobacillus acidophilus*, corynebacteria, peptostreptococci, staphylococci, streptococci and bacteroides predominate. After menopause, the pH rises again, less glycogen is secreted and the flora returns to that found in prepubescent females [19].

Yeasts are occasionally found in the vagina of about 10 – 30% of women, these sometimes increase and cause vaginitis [20]. In the anterior urethra of humans, *S. epidermidis*, enterococci and diphtheroids are found frequently, while non-pathogenic species such as *E. coli*, proteus, pseudomonas and neisseria are reported occasionally [20].

Methodology

Study Design

The study was experimental, assessing the effects of antifungal vaginal creams on the microflora of the vagina. The antifungal creams were: CZ which contained 50mg clotimazole, 1% Benzyl alcohol per 5g of gel; MS, GZ and NS which contained 10mg clotrimazole, 0.275% Nipastat, 0.30% Imidurea per 1g of gel; CS which contained 10mg clotrimazole, 1% Benzyl alcohol. Different micro-organisms which are commonly found as microflora of the vagina were collected and tested against the five different antifungal creams above, which are commonly used in Namibia. The organisms included *L. acidophilus, Staphylococcus epidermidis, Staphylococcus aureus, Streptococcus agalactiae, Streptococcus pyogenes, Klebsiella Pneumoniae, Escherichia coli* and *Proteus aeruginosa*. The bacterial isolates were collected from the Namibia Institute of Pathology (NIP), microbiology laboratory. The identification of the isolates was done using the VITEK® 2 Compact, bioMérieux instrument Durham, New York).

Antimicrobial Susceptibility Testing

Testing for the antibacterial activities of each cream was carried out using agar well diffusion method proposed by Ratnamma et al., 2009 [21]. The inoculums were cultured on blood agar and incubated for 24 hours at 37ºC. After incubation, a suspension equivalent to 0.5 McFarland Standard was prepared from each plate. A sterile swab was used to evenly distribute the lawn of culture on Mueller Hinton agar plates. The plates were allowed to dry for 15 minutes before they were used in the test.

Wells were then created on the agar and a sterile swab was used to fill up the well with the antifungal cream on each plate. Each isolate was subjected to five antifungal vaginal creams. The plates were left to stand for 1 hour to allow diffusion to take place and were incubated at 37ºC and measurements taken at intervals of 24 hours, 48 hours and 72 hours for a zone of inhibition around the well, a transparent ruler was used to measure the inhibition zones as described in the method by Delahaye et al., 2009 [22].

To ensure reliability, the entire experiment was done in duplicates for each isolate against each cream and a third run was done for the results that were contradicting. *Candida albicans* was subjected to all five antifungal vaginal creams and was used as a positive control, to confirm that creams possess antifungal effects. Furthermore, antibiotic discs such as vancomycin were used as a positive control for *S. aureus, S. epidermidis* and *S. agalactiae*. Gentamicin was used for *K. pneumoniae*, *E.coli* and *P. aeruginosa*. Erythromycin was used for *S. pyogenes* and Penicillin for Lactobacillus species. Un-inoculated Muller Hinton plates were used as negative controls.

Methods of Data Analyses

Data was analysed using Microsoft Excel. Results were entered in tables which displayed the test isolates and antifungal vaginal creams they were subjected to, and the measurements of the diameter for the zones of inhibition.

Ethical Considerations

Authorization for conducting this study was granted by the Ministry of Health and Social Services (MoHSS), Namibia Institute of Pathology and Namibia University of Science and Technology Research and ethics committees.

Results

The antimicrobial activities of the antifungal vaginal creams against eight bacterial isolates were assessed by measuring the zone of inhibition around the antifungal cream at intervals of 24, 48 and 72 hours.

Table 1: Effects of the antifungal creams on microorganisms commonly found as vaginal flora.

	Zone of inhibition of antifungal vaginal creams (present/absent, after 24 hour incubation)				
Organism	CZ	CS	GZ	MS	NS
Lactobacilli	Absent	Absent	Absent	Absent	Absent
S. epidermidis	Absent	Present	Present	Present	Present
S. aureus	Absent	Present	Present	Present	Present
E. coli	Absent	Absent	Absent	Absent	Absent
K. pneumoniae	Absent	Absent	Absent	Absent	Absent
P. aeruginosa	Absent	Absent	Absent	Absent	Absent
Streptococcus agalactiae	Absent	Absent	Absent	Absent	Absent
S. pyogenes	Absent	Absent	Absent	Absent	Absent

All antifungal creams, except CZ exhibited antibacterial effects against staphylococcus strains used.

Table 2: Organisms mostly affected by antifungal vaginal creams.

	Zone of inhibition on antifungal vaginal creams after 24 hours incubation				
Vaginal flora	CZ	CS	GZ	MS	NS
S. epidermidis	Absent	Present	Present	Present	Present
S. aureus	Absent	Present	Present	Present	Present

S. epidermidis and *S. aureus* were the only vaginal flora affected by the antifungal vaginal creams.

Table 3: Antimicrobial activity of the creams against vaginal flora.

	Zone of inhibition (diameter, mm) of antifungal vaginal creams after 24 hours incubation				
Organism	CZ	CS	GZ	MS	NS
Lactobacilli	0	0	0	0	0
S. epidermidis	0	3	4	3	3
S. aureus	0	3	4	2	3
E. coli	0	0	0	0	0
K. pneumoniae	0	0	0	0	0
P. aeruginosa	0	0	0	0	0
S.agalactiae	0	0	0	0	0
S. pyogenes	0	0	0	0	0

GZ had a greatest effect on both *S. epidermidis* and *S. aureus* (4mm).

Table 4: Effects of prolonged application of the creams on the microflora of the vagina.

Vaginal Microflora	Zone of inhibition (mm, diameter) of antifungal vaginal creams														
	CZ			CS			GZ			MS			NS		
Time (Hours)	24	48	72	24	48	72	24	48	72	24	48	72	24	48	72
Lactobacilli	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S. agalactiae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S. pyogenes	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
E. coli	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
K. pneumoniae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
P. aeruginosa	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S. epidermidis	0	0	0	3	3	3	4	4	4	3	3	3	3	3	3
S. aureus	0	0	0	3	3	3	4	4	4	2	2	2	3	3	3

No cream exhibited antimicrobial activity due to prolonged incubation.

Discussion

The vagina of a healthy fertile woman harbours different microbiota that can be grouped into transient and resident flora. Microorganisms differ in their nutritional requirements and the level of susceptibility to antimicrobial agents [23].

The inhibition of the growth pattern of the isolates indicates the varying abilities of the organisms to resist the antimicrobial effects of the creams. However, these variations could be due to the differences in the nature and structures of the bacterial cell wall, which is regarded as the ultimate target of any antimicrobial agent [24]. The results obtained in this study revealed that GZ, CS, MS and NS exhibited antimicrobial activity, which is the ability of the cream to inhibit the growth of tested organisms as shown in table 1. This was qualitatively assessed by observing for the presence or absence of the inhibition zone.

The active ingredient in the cream distinguishes one type of cream from another. Clotrimazole is the active ingredient in all the antifungal creams used in this study [13]. Table 2 indicates that the creams possessed antimicrobial activity against *Staphylococcus aureus* and *Staphylococcus epidermidis*. However, it was observed that CZ did not exhibit any antimicrobial

activity against these test organisms. Clotrimazole acts against the Gram-positive organisms by distorting the peptidoglycan, which is a layer that constitutes the cell wall of all Gram-positive organisms [25]. The activity of the creams against staphylococcal organisms is therefore attributable to clotrimazole present in the creams.

Despite clotrimazole being active against Gram-positive organisms, it did not exhibit any antibacterial effects against some of the Gram-positive organisms (Lactobacillus, *S.pyogenes* and *S.agalactiae*) used in the study as shown in table 3. This could be due to the fact that the concentration of clotrimazole present in the creams may not be sufficient to exhibit effects against these Gram-positive bacteria. There were no observed inhibitory effects against *E. coli, K. pneumoniae*, and *P. aeruginosa*. These organisms being Gram-negative, they have little peptidoglycan in their cell wall. However, they contain a unique component, lipopolysaccharide [25]. Therefore, since the cell wall is not exposed, this may hinder the activity of clotrimazole, which is the active component in the creams.

The resistance of these organisms to antimicrobial agents is usually due to chromosomal mutation which lowers the permeability of the bacteria to the agent or the acquisition of resistance plasmids and transposons [24]. Therefore the resistance showed by *E. coli, K. pneumoniae* and *P. aeruginosa* may be due to chromosomal mutation.

The findings of this research are in agreement with findings of a similar study conducted by Rossa et al which assessed in-vitro effects of clotrimazole on *Lactobacillus acidophilus*, *S. epidermidis*, *Provotella bivia* and group D streptococcus species grown in a continuous culture medium [26]. Results from their study indicated that treatment with clotrimazole had deleterious effects on normal vaginal flora, as it altered components of the vaginal flora, without returning to normal.

Findings of this study also agree with results of a study done by Liss et al which compared the in vitro activity of fluconazole and clotrimazole. The results indicated that clotrimazole exhibited bactericidal effects against lactobacillus and streptococcus, which means clotrimazole has the ability to change the ultrastructure of bacteria.

On the contrary, a study done by Boag et al on patients with culture positive and symptomatic vaginal candidiasis in Genitourinary medicine clinic at St Stephan's hospital in London found no significant difference in the vaginal flora after treatment with clotrimazole-containing antifungal creams [27]. Their results agree with those of Neat et al who found no significant effects of clotrimazole on vaginal flora [28].

However, Neut et al further enlightened that there may not be a correlation between the in vitro and in vivo effects of antifungal vaginal creams on the normal vaginal flora. This is because in vivo, factors such as combination of clotrimazole with other preservatives inhibits growth of one or more strains of the normal flora that can be found on the vaginal mucosa.

The effects of antifungal creams against all selected isolates used in the study over a prolonged incubation time showed no significance, as the zone of inhibition remained unchanged after 24 hours, 48 hours and 72 hours for each cream against each isolate as shown in table 4.

The antimicrobial activity of antifungal vaginal creams on the vaginal flora may also be due to the combination of the ingredients. From this study, antifungal vaginal creams which exhibited antimicrobial activity contain a mixture of 0.275% Nipastat and 0.30% Imidurea (Biopure 100). These ingredients are used as preservatives in pharmaceuticals and provide a broad spectrum of activity against growth of molds, bacteria and yeast [29]. Furthermore, they act by interfering with microbial growth, multiplication and metabolism. They also alter the bacterial cell membrane's permeability, causing leakage of cell constituents, complete lysis and leakage of cytoplasmic constituents [29]. Benzyl alcohol (1%) used as a preservative for CZ also has the ability to suppress growth of bacteria by interfering with protein synthesis [30].

Conclusion

Certain topical antifungal vaginal creams may interfere with the growth of normal vaginal flora causing vaginal flora imbalance. Drugs for treatment of candida vaginitis should contain a broad spectrum of antimycotic activity and should exhibit no effects against normal flora of the vagina. This will enhance faster and more reliable treatment. The results from the study indicated that CS, GZ, MS and NS vaginal creams possesses antimicrobial activity against vaginal microflora.

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