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Effectiveness and Pharmacokinetic evaluation of Geonistin® (Oxytetracyline and Nystatin) Vaginal Tablets for Unspecific and Mixed Vulvovaginal Infections (GENIE Study)

Višnja Škerk[1], Adela Kolumbić Lakoš[2], Maja Gašparić[3], Mirjana Matrapazovski Kukuruzović[2], Ivana Magdić Blažević[2]

[1]Department for Urogenital infections, Dr. Fran Mihaljević University Hospital for Infectious Diseases, Zagreb, Croatia; [2]PLIVA Croatia LTD, Zagreb, Croatia; [3]Teva Pharmaceuticals Europe BV, Amsterdam, Netherlands

Corresponding author:
Mirjana Matrapazovski Kukuruzović, MD
PLIVA Croatia LTD
Prilaz baruna Filipovića 25
10000 Zagreb
Croatia
mirjana.matrapazovski-kukuruzovic@pliva.com

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ABSTRACT The GENIE study was performed to evaluate the effectiveness and systemic exposure to oxytetracycline in local treatment of unspecific and mixed vulvovaginal infections characterized by vaginal discharge with Geonistin® vaginal tablets (100 mg oxytetracycline and 100 000 IU nystatin). The total number of subjects enrolled was 189. The treatment had beneficial effects in 100% of the study population. According to the Nugent score, the treatment had a positive effect in 89.2% of participants. The microbiological cure rate was 78.8%. Oxytetracycline concentration levels were from 13.3 to 32.2 ng/mL in 11 out of 15 subjects, and in four subjects the levels were below 10 ng/mL. Geonistin® had a beneficial effect on the unspecific and mixed vulvovaginal infections characterized by vaginal discharge in all efficacy and safety outcomes. Microbiological and the Nugent score efficacy measures confirmed clinical effectiveness. Beneficial efficacy results were achieved with only a few non-serious adverse events.

KEYWORDS: oxytetracycline, nystatin, vaginal discharge, vaginitis, vulvovaginitis, pharmacokinetics

INTRODUCTION

Geonistin® is a drug containing oxytetracycline and nystatin in vaginal tablet form (1). Geonistin® contains 100 mg oxytetracycline and 100 000 IU nystatin per vaginal tablet (1). It was registered for the first time in April 1964 in former Yugoslavia by PLIVA. Geonistin® was extensively used for the local treatment of vulvovaginitis for almost half a century. However, despite long-term use there was a substantial lack of data acquired in clinical studies.

Oxytetracycline is a broad-spectrum antibiotic active against a wide variety of bacteria, and nystatin is a polyene antifungal medication. Geonistin® vaginal tablets are used for the local treatment of vaginitis and cervicitis caused by *Candida albicans* as well as other fungi and bacteria sensitive to oxytetracycline and nystatin (1). Geonistin® vaginal tablets are also used for prophylaxis of vaginitis and cervicitis after gynecologic procedures (e.g. cauterization) (1). They

are also used for prophylaxis of fungal superinfection in bacterial inflammation of mucous membrane of the vagina (1). Oxytetracycline and nystatin were used in the form of various vaginal formulations for more than 50 years in the treatment of non-trichomonal vaginal discharge, either as individual compounds or as fixed combinations (2). Treatment with Geonistin® in standard clinical practice lasts for six consecutive days and can be extended up to 12 days. In the post-marketing period, it was perceived as an effective and well-tolerated drug. The most commonly reported side-effect of Geonistin® was vaginal irritation, occurring very rarely (<1/10 000) (1). Based on clinical experience, Geonistin® has low toxicity and is well tolerated. Unknown risks and side-effects are possible, so additional studies are required. Additionally, there is almost no available data on efficacy and safety as well as systemic bioavailability after vaginal administration of oxytetracycline. It is known that 40-80% of tetracyclines are bound by serum (3). Oxytetracycline is classified as short-acting compound based on serum half-lives of 6-8 hours (3). Beside Geonistin®, several other topical administration drugs are available (4-6). Data on effectiveness and safety for a variety of other systemic (oral) or topical (vaginal) treatments are available. However, these treatments target primarily mycotic or primarily bacterial causative agents (7-9). That is why they were not considered appropriate comparators in the treatment of study indications and the study was performed as a non-comparative study.

Most common vulvovaginal infections characterized by vaginal discharge include bacterial vaginosis (BV), vulvovaginal candidiasis (VVC), trichomoniasis, and aerobic vaginitis, and vaginal discharge can occasionally be caused by *Neisseria gonorrhoeae* and *Chlamydia trachomatis* infection (10-12). The majority of women with vaginal infection (13) report symptoms such as discharge, itching, or odor (14). Although in everyday clinical practice a gynecologist may differentiate between these conditions based on characteristics of vaginal discharge and some specific signs and symptoms (15), conditions implied by the term "vulvovaginal infections characterized by vaginal discharge" cannot always be clinically distinguished nor are they recognized or defined by separate ICD-10 codes (11). Diagnostic methods such as wet preparation are rarely performed in daily clinical practice. PAP smear or microbiological evaluation are performed in daily clinical practice, but not for the diagnosis of vaginal discharge. The term "non-specific vulvovaginal infection" is frequently used for vulvovaginal infections, as physicians are not able to define the condition using the abovementioned terminology. Furthermore, a number of cases are characterized as mixed vaginal infection, which refers to the finding of mixed bacterial pathogens or mixed bacterial and fungal pathogens (16,17). In everyday clinical practice, patients with vulvovaginal discharge are treated based on the experience of the physician.

MATERIALS AND METHODS

This study was performed to evaluate the effectiveness as well as the systemic exposure to oxytetracycline in local treatment of vulvovaginal infections with Geonistin® vaginal tablets. It was conducted as prospective, non-comparative, interventional, phase 4 study with pharmacokinetics (PK) evaluation. The study was conducted from September to December 2016 in 15 investigation sites in Croatia and one satellite site responsible for PK assessment and analysis. Patients with unspecific and mixed vulvovaginal infections characterized by a vaginal discharge that required local treatment were included. The diagnosis was based on clinical, microscopic, and culture findings. The patients also self-evaluated signs and symptoms (presence/absence) as well as their satisfaction at the end of the study. Patients were included only if they meet all inclusion and exclusion criteria predefined by the study protocol. Inclusion criteria were: Diagnosis of unspecific and mixed vulvovaginal infections characterized by vaginal discharge (pathological and/or unusual) and at least one additional sign/symptom; Women of reproductive age, over 18 years of age; Lactobacillary grade ≥2 in vaginal exam; According to the investigator's judgment, the patients should receive local treatment only; Written informed consent obtained and signed; Patients in good health as determined by medical history, medical examination; Women of childbearing potential (not surgically sterile or postmenopausal for over 2 years) using a medically accepted method of contraception and agreeing to continue use of this method for the duration of this study and for 30 days after discontinuation of the study drug. Acceptable methods of contraception include hormonal contraceptive (oral, implanted, transdermal, or injected), abstinence, and partner vasectomy or must have exclusively same-sex partners (exclusively homosexual relationships); The patient must be willing and able to comply with study restrictions and be willing to return to the clinic for the Visit 2 evaluation as specified in the study protocol. Exclusion criteria were: *Trichomonas vaginalis* infection (detected by wet preparation of vaginal smear or confirmed by OSOM® rapid test); *Neisseria gonorrheae* infection (clinically suspected); *Chlamydia trachomatis* infection (clinically suspected); Infection with *her-*

pes simplex virus (clinically suspected); Patients with other vaginal or vulvar condition which would confound the interpretation of clinical response; Women with cervical carcinoma; Women under treatment for cervical intraepithelial neoplasia (CIN); Recurrent BV (three or more episodes of BV in 12 months); Recurrent VVC (four or more VVC episodes in 12 months); Use of a topical or systemic antimicrobial treatment within 30 days before the study; Ongoing immunosuppressant treatment; Ongoing systemic antimicrobial therapy; Ongoing use of vaginal probiotics (oral and vaginal tablets and all other form which help regeneration of vaginal flora); Women currently menstruating or expecting menstruation within 1 week; Women using an intrauterine contraceptive device; Pregnancy (excluded by pregnancy urine test) or plans to become pregnant during the study; Lactation; Patients who gave birth or underwent gynecological surgery within 2 months prior to the study; History of allergic reactions to nystatin or oxytetracycline or to excipients of the pharmaceutical product; Participation in another clinical study; The patient being unwilling or unable to administer study drug.

Participants were recruited by physicians from their everyday practice during regular office visits (Visit 1/ Baseline – V1). The sample size was 192 women, including 15 patients for PK measurements. The estimated dropout rate was 20%. Geonistin® vaginal tablets were administered in line with the approved posology (1). One vaginal tablet containing 100 mg oxytetracycline and 100 000 IU nystatin was self-administered by patients once daily, between 21:00 h and 24:00 h over six consecutive days. The primary study objective was to assess the clinical effectiveness of 6-day treatment with Geonistin® vaginal tablets in the empirical treatment of vulvovaginal infections at Day 13±2 (Visit 2 – V2). If a patient came to the second visit before 13±2 days this was considered an early termination date (ET). The secondary objectives were to determine systemic exposure to oxytetracycline in local treatment of vulvovaginal infections; Cure rate according to the Nugent score (18); Microbiology and microscopy results (outcome); and to evaluate safety throughout the study. Effectiveness evaluation using Nugent score was applied for patients with a baseline Nugent score ≥4. Samples for evaluation were taken at Visit 2/Early termination date (V2/ET). Cure was classified as follows: Nugent score 0-3; Improvement in Nugent score >3, but improved by at least 1 point over the baseline; or failure: no improvement in Nugent score.

Three analysis datasets (populations) were used in the study protocol: The Safety population, the Intention to Treat (ITT) and the Per Protocol (PP) populations. All enrolled patients were included in the Safety population. The ITT population consisted of all enrolled patients who received at least one dose of study medication. The PP population consisted of all enrolled patients who received all six doses of the study medication, without violation of any of the inclusion and exclusion criteria or Visit 2 date (the 13±2 days specified in protocol or later if patient had the menstrual period on day 13±2, which was an acceptable condition for delay according to the study protocol Amendment 01), and with valid data on the primary variable.

All required data and three swab samples were firstly collected at Visit 1 on Day 1. One swab was used for wet mount preparation of vaginal smear and microscopy preparation colored by Gram, the second one was used for microbiological testing (Amies), and the third one was used for exclusion of *Trichomonas vaginalis* infection by the OSOM® Trichomonas Rapid Test (19). Required data and two samples were then collected at Visit 2/ET at Day 13±2. One swab was used for wet mount preparation of vaginal smear and microscopy preparation colored by Gram, the second one was used for microbiological testing (Amies). The Central Lab was used for Nugent score and microbiological testing. Evaluation of microbiological outcomes was performed using grading based on the four-quadrant semi-quantitatively scoring method. Numbers of grown colonies were quantified as 1+, 2+, 3+, and 4+. Samples for evaluation were taken at Visit 2/ET and the outcome was defined as Microbiological cure: A patient with negative culture (no growth) for baseline pathogen or result 1+ (if baseline result was >1+); Microbiological improvement: Improvement in semi-quantitative evaluation over the baseline (but does not meet criteria for microbiological cure); or Microbiological failure: A patient with positive culture for baseline pathogen with no improvement in semi-quantitative evaluation.

A subset of patients that underwent the pharmacokinetic testing included the first 15 patients from sites located in Zagreb that accepted participation. All sites were notified to stop further inclusion of patients for pharmacokinetic testing after 15 patients were accepted and performed blood sampling. The selected 15 patients from sites located in Zagreb were invited to come on the morning after the last dose of Geonistin® was applied (Day 7) for pharmacokinetic measurement of oxytetracycline. A peripheral venous blood sample was taken in order to determine steady-state serum concentrations of oxytetracycline. The Central Lab was used for pharmacokinetic measurement.

Efficacy analyses were carried out on the ITT population as well as in PP population. Safety analysis, demographics, and baseline characteristics were evaluated in the Safety population. For continuous variables, descriptive statistics: number (n), mean, standard deviation, median, minimum, and maximum were provided. For categorical variables, patient counts (n) and percentages (%) were provided. We used analysis of variance (ANOVA), Pearson's Chi-square, or Fisher's exact test if cell sizes were too small. Missing categories were presented if necessary. Sufficient power (0.8) for the desired effect size and significance level (0.05) was applied using a 95% confidence interval (CI). Appropriate statistical tests were performed. The allowed Type I error was 0.05. *P*-values less than 0.001 were reported as <0.001. Statistical analysis was performed using SPSS 19.0; Microsoft MSWord 2010 was used for reporting.

RESULTS

We screened 194 subjects at 15 investigation centers in Croatia, out of which 2 were screening failures. The total number of subjects who were enrolled in the study was 192 (Safety population). Of these subjects, 189 received at least one dose of the study medication (ITT population) (Figure 1). Only three subjects failed to return for Visit 2 assessment. Of these 189 patients, 7 attended Visit 2 outside the time window allowed in the protocol (the 13±2 days specified in the protocol or later if the patient had the menstrual period on Day 13±2). 2 subjects were enrolled as per protocol, but they were excluded from the study for using the concomitant medication which was forbidden or described as exclusion criteria. A total of 177 patients were in the PP population. 25 subjects performed Visit 2 at a later time, but their reasoning for the delay was a menstrual period (an acceptable condition for the delay). Subjects who did not use all six vaginal tablets were not included in the PP population because the primary effectiveness measure and time point for this study was clinical cure after six days of therapy. The reasons for discontinuation of the therapy were menstrual bleeding for two subjects and an adverse event for one subject (Figure 1).

Demographics, baseline characteristics, total signs and symptoms

The mean age of all enrolled subjects was 32.6 years. There was no significant difference in the distribution of subjects by age in the four diagnostic groups. The distribution of subjects in the four baseline diagnosis groups in the three study populations – ITT, PP, and Safety – are shown in Table 1. Descriptive statistics and ANOVA of age by diagnosis were not significant. Descriptive statistics of baseline demographic data (height/weight/BMI) are shown in Table 2. No significant difference was found between the vital sign measurements and the physical examination (height (cm), weight (kg), BMI (kg/m^2)) between the two study visits. The sum of individual objective vulvovaginal signs and subjective symptoms was evaluated at both study visits. The mean change from Visit 1 to Visit 2 was -5.87, indicating significant overall treatment effect. The minimum change was at least one score. The significance level of change from baseline of total signs and symptoms score was $P<0.001$.

Efficacy results – PP data

The clinical cure rate was assessed for the 177 study subjects belonging to the PP population. The clinical cure rate after six days of treatment was 149 (84.2%), and clinical improvement was observed in an additional 28 (15.8%) of the patients. There was no subject for whom the treatment failed; hence the treatment had beneficial effects in 100% of the study population. The clinical cure rate for the PP population was 84.2%, 95% CI (0.780-0.892), $P<0.001$. The clinical cure rates were ≥80% in all diagnostic groups, with the highest rate in the "Candidiasis" group at 91.3%, 95% CI (0.72-0.99). The hypothesis test result ($P<0.05$ in all diagnostic groups) provided evidence that the treatment can achieve cure in the PP population. Clinical effectiveness and cure rate by diagnosis group in the PP population is shown in Table 3.

Table 1. Distribution of subjects in the four baseline diagnosis groups in the three study populations: Intention to Treat (ITT), Per Protocol (PP), and Safety

	Candidiasis (n=27)	Bacterial infection (n=89)	Mixed infection (n=27)	Clinically significant microorganisms not isolated (n=49)	Total (N=192)
ITT, n (%)	26 (13.8 %)	87 (46.0%)	27 (14.3%)	49 (25.9%)	189 (100.0 %)
PP, n (%)	23 (13.0%)	85 (48.0%)	25 (14.1%)	44 (24.9%)	177 (100.0 %)
Safety, n (%)	27 (14.1%)	89 (46.3%)	27 (14.1%)	49 (25.5%)	192 (100.0 %)

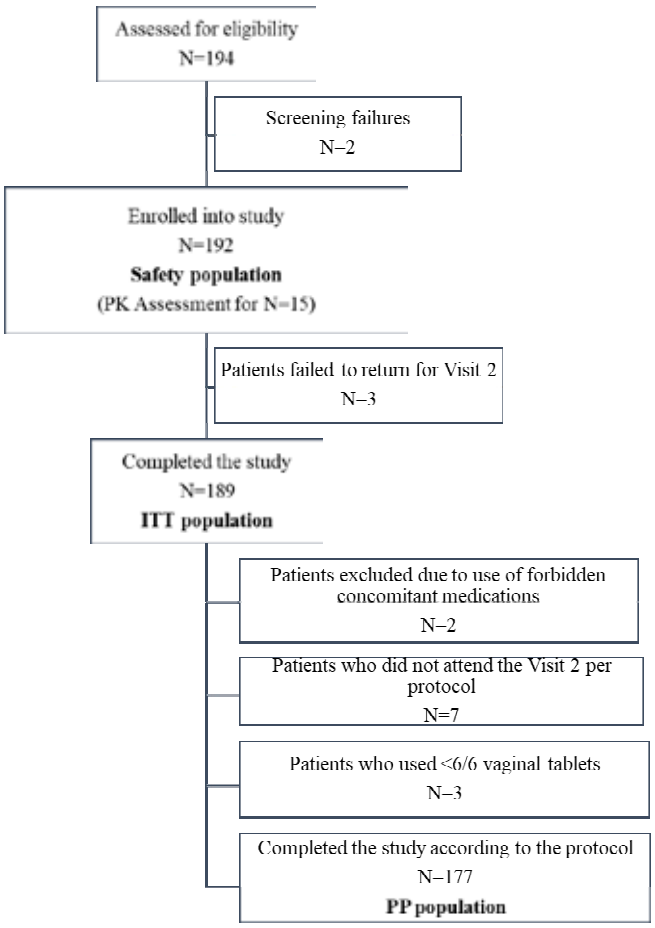

Figure 1. Disposition of subjects.

Table 2. Baseline demographic data

Height, Weight, BMI	Candidiasis	Bacterial infection	Mixed infection	Clinically significant microorganisms not isolated	Total
N	27	89	27	49	192
Age (years)*					
Mean (SD)	31.2 (7.85)	33.2 (8.64)	34.1 (7.52)	31.6 (7.52)	32.6 (8.1)
Median	27.5	32.5	34.8	33.1	32.8
Min-Max	21.8-50.1	18.6-55.5	20-46.7	18.2-47.2	18.2-55.6
Height (cm)					
Mean (SD)	165.5 (5.09)	167.0 (5.99)	166.6 (5.63)	167.0 (5.41)	166.8 (5.67)
Median	166	167.5	167	167	167
Min-Max	156-177	150-180	153-176	157-178	150-180
Weight (kg)					
Mean (SD)	57.9 (7.42)	62.4 (9.97)	61.2 (9.17)	63.8 (11.0)	62.0 (9.92)
Median	56	61	60.7	60.5	60
Min-Max	49 - 76	45.5 - 105.5	42 - 85	48 - 101	42 - 105.5
BMI (kg/m²)					
Mean(SD)	21.1 (2.14)	22.2 (3.15)	22.1 (2.75)	23.3 (4.25)	22.3 (3.36)
Median	20.8	21.7	22.0	22.5	21.6
Min-Max	17.6-26.0	17.3-33.3	17.9-29.8	17.2-36.7	17.2-36.7
* Significance level of treatment group comparison: *P*=0.403					

SD: Standard Deviation; BMI: body-mass index

Table 3. Clinical effectiveness, cure rate by diagnosis group – Per Protocol (PP)

Diagnosis group		Frequency	Percent	CI	*P*-value
Candidiasis	Cure	21	91.3	[72.0%, 98.9%]	*0.001*
	Improvement	2	8.7		
	Total	23	100.0		
Bacterial infection	Cure	73	85.9	[76.6%, 92.5%]	*<0.001*
	Improvement	12	14.1		
	Total	85	100.0		
Mixed infection	Cure	20	80.0	[59.3%, 93.2%]	*0.029*
	Improvement	5	20.0		
	Total	25	100.0		
Clinically significant microorganisms not isolated	Cure	35	79.5	[64.7%, 90.2%]	*0.006*
	Improvement	9	20.5		
	Total	44	100.0		

Efficacy results – ITT data

The primary efficacy analysis was repeated on the 189 study subjects belonging to the ITT population to assess whether non-compliance had any effect on the results obtained for the PP population. The clinical cure rate after six days of treatment was 154 (81.5%), and clinical improvement was observed in another 35 (18.5%) of the patients. There was no subject for whom the treatment was not clinically effective; hence the treatment had beneficial effects in 100% of the study population, as shown in Table 3 and Table 4. The clinical cure rate with 95% CI (0.75-0.87), $P<0.001$ satisfied the condition for effectiveness. The clinical cure rate was similar in the four diagnostic groups. Clinical effectiveness and cure rate by diagnosis group in the ITT is shown in Table 4.

Cure rate according to the Nugent score, which was evaluated for all ITT patients at Visit 2/ET: of the 189 subjects who finished the study 51 had Baseline Nugent score below 4 and 138 had quantifiable results above 4, which was used for assessment of efficacy. The treatment had a positive effect in 123 of the 138 subjects (89.2%) and no effect in 15 (10.8%).

38 of the 189 ITT subjects had no clinically significant microorganisms isolated either at Visit 1 or Visit 2. Therefore, microbiological cure rate could only be assessed for the remaining 151 subjects, where the microbiological cure rate was 78.8%. Improvement was observed in additional four subjects, and treatment failed only in the case of 4 subjects (less than 6% of the study population). The cure rate was above 74% in all three diagnosis groups. In 24 subjects (15.9%) a "new infection" occurred at Visit 2, which was not previously detected. Microbiological cure rate for the ITT population and microbiological cure rates for subjects with "new infection" are shown in Table 5.

Analysis of the effectiveness based on microbiological results by diagnostic group found that the microbiological cure rates for subjects with isolated

Table 4. Clinical effectiveness, cure rate by diagnosis group – Intention to Treat (ITT)

Diagnosis group		Frequency	Percent	CI	*P*-value
Candidiasis	Cure	22	84.6	[65.1%, 95.5%]	*0.009*
	Improvement	4	15.4		
	Total	26	100.0		
Bacterial infection	Cure	74	85.1	[75.8%, 91.8%]	*<0.001*
	Improvement	13	14.9		
	Total	87	100.0		
Mixed infection	Cure	21	77.8	[57.7%, 91.4%]	*0.046*
	Improvement	6	22.2		
	Total	27	100.0		
Clinically significant microorganisms not isolated	Cure	37	75.5	[61.1%, 86.7%]	*0.019*
	Improvement	12	24.5		
	Total	49	100.0		

Table 5. Microbiological cure rate – Intention to Treat (ITT)

Change in the status	Frequency	Percent
Microbiological cure rate – ITT		
Cure	119	78.80%
Improvement	4	2.65%
Failure	4	2.65%
New infection	24	15.90%
Total	151	100.0%
Subjects with "Clinically significant microorganisms not isolated" at V1 nor V2, N=38		
Microbiological cure rate for subjects with "new infection" for microorganisms isolated at Visit 1 – ITT		
Cure	10	41.7%
Improvement	2	8.3%
Failure	1	4.2%
No clinically significant microorganism isolated at V1	11	45.8%
Total	24	100.0%

microorganisms at V1 ranged from 74.1% for the "Mixed infection" group up to 88.5% in the "Bacterial infection" group. Microbiological cure rates by diagnosis group in the ITT population are shown in Table 6. Microbiological cure rates were high for all isolated pathogens except for *Proteus mirabilis*, for which microbiological outcome failed. The microorganisms that were most commonly isolated in the ITT population were *Gardnerella vaginalis*, *Candida albicans*, and *Enterococcus faecalis* with cure rates 98.5%, 90.5%, and 88.0%, respectively. Microbiological effectiveness for isolated pathogen in the ITT population is shown in Table 7.

Bioanalytical pharmacokinetics evaluation

Steady-state serum concentrations of oxytetracycline were calculated from PK measurements performed on Day 7. Of the 15 subjects, 4 had oxytetracycline concentration levels below 10 ng/mL (Limit of Quantification: LOQ=10). As these values were not quantifiable, additional sensitivity analysis was performed with the following three scenarios being investigated: (a) Non-quantifiable concentration levels substituted by the LOQ/2 value; (b) Non-quantifiable concentration levels substituted by the limit value LOQ; (3) Non-quantifiable concentration values left out of the analysis. Descriptive statistics of oxytetracycline concentrations – ITT are shown in Table 8.

Adverse events

Only 15 adverse events (AE) occurred during the study, with 13 patients being affected in total. One pregnancy was reported. Urinary tract infection was reported in 2 study patients, while all other AEs occurred only once. Of the total 15 AE, none were serious, while just one (vulvovaginal discomfort) was reported to be probably related to the study drug. No AE with increased severity and no serious AE were reported throughout the study. No deaths occurred during the study.

Table 6. Microbiological cure rate by diagnosis group – Intention to Treat (ITT)

Diagnosis group	Microbiological result at V2	Frequency	Percent
Candidiasis (n=26)	Cure	22	84.6%
	Improvement	1	3.9%
	Failure	1	3.9%
	New infection	2	7.6%
	Total	26	100.0%
Bacterial infection (n=87)	Cure	77	88,5%
	Improvement	0	0%
	Failure	3	3.4%
	New infection	7	8.1%
	Total	87	100.0%
Mixed infection (n=27)	Cure	20	74.1%
	Improvement	3	11.1%
	Failure	0	0%
	New infection	4	14.8%
	Total	27	100.0%
Clinically significant microorganisms were not isolated (n=49)	Clinically significant microorganisms were not isolated	38	77.6%
	New infection	11	22.4%
	Total	49	100 %

Table 7. Microbiological effectiveness for isolated pathogen – Intention to Treat (ITT) population

Pathogen isolated at V1	Microbiological effectiveness			
	Cure	Improvement	Failure	Total
Gardnerella vaginalis	66 (98.5%)	1 (1.5%)	0 (0%)	67 (100%)
Candida albicans	48 (90.5%)	3 (5.7%)	2 (3.8%)	53 (100%)
Enterococcus faecalis	22 (88.0%)	1 (4.0%)	2 (8.0%)	25 (100%)
Escherichia coli	12 (85.7%)	2 (14.3%)	0 (0%)	14 (100%)
Streptococcus agalactiae (BHS-B)	13 (100.0%)	0 (0%)	0 (0%)	13 (100%)
Staphylococcus aureus	3 (100.0%)	0 (0%)	0 (0%)	3 (100%)
Proteus mirabilis	0 (0%)	0 (0%)	1 (100.0%)	1 (100%)
Klebsiella pneumonia	1 (100.0%)	0 (0%)	0 (0%)	1 (100%)
Sphingomonas spp.	1 (100.0%)	0 (0%)	0 (0%)	1 (100%)

Adherence and patient satisfaction

Patient adherence was reported as follows: of the 189 subjects who completed the study, 186 (98.4%) used all six tablets, one subject (0.5%) used only 5, while the remaining two subjects (1.1%) used less than 5. The large majority of study subjects were very satisfied with the study drug: 108 (57.2%) of them considering it to be excellent and another 48 (25.4%) very good. A further 25 (13.2%) found it good, with only four subjects (2.1%) considering it satisfactory and another four (2.1%) not satisfactory. Of the eight subjects indicating satisfaction level as "Satisfactory" or "Not satisfactory" only one subject had clinical improvement and the remaining seven were clinically cured, so subject satisfaction level was not related to clinical efficacy. Subject satisfaction level with the study drug was similar in the four disease groups.

DISCUSSION

This prospective non-comparative study evaluated the effectiveness and safety of Geonistin® in the local treatment of unspecific and mixed vulvovaginal infections characterized by vaginal discharge. According to the European guideline for the management of vaginal discharge, accurate diagnosis is important for the selection of appropriate specific treatment (20), however, in clinical practice this is not often possible. The etiological variety of vaginitis indicates the use of broad-spectrum treatment as first-line therapy and performing a microbiological analysis if therapy is not successful (21). As expected, and in line with previous clinical experience, Geonistin® vaginal tablets showed excellent efficacy results. The clinical cure rate was high, 81.5% for the ITT population and 84.2% for the PP population. The study provided evidence of Geonistin® effectiveness in patients with unspecific and mixed vulvovaginal infections characterized by vaginal discharge.

The clinical cure rates for Geonistin® were similar in all four diagnostic groups, with the highest rate (85.1%) in the "Bacterial infection" group. Furthermore, microbiological cure was achieved in 78.8% participants. Subjects not classified as clinically cured by the vaginal tablets all belonged to the clinical improvement group, and no subject failed the trial. Geonistin® was well tolerated considering only 15 AE were reported during the study in 13 patients, and none of them were serious. Only one event (vulvovaginal discomfort) was categorized by the investigator as related to the study drug. However, vulvovaginal discomfort was already described as AE (1). Since most women will experience vaginal infection during their lifetime with uncomfortable symptoms, it is important to have an effective and well tolerated drug (14). Moreover, Geonistin® demonstrated the ability to significantly decrease the total scores of vulvovaginal signs and symptoms.

Of the 15 subjects who were selected to PK measurement, 11 subjects had oxytetracycline concentration levels from 13.3 ng/mL to 32.2 ng/mL and four subjects had oxytetracycline concentration levels below 10 ng/mL. To the best of our knowledge, this

Table 8. Oxytetracycline serum concentrations – Intention to Treat (ITT)

	n	Mean (ng/mL)	CI for mean	SD	Median (ng/mL)	Min (ng/mL)	Max (ng/mL)
1.	15	16.12	[11.62, 20.62]	8.12	17.60	5.00	32.20
2.	15	17.45	[13.98, 20.93]	6.28	17.60	10.00	32.20
3.	11	20.16	[16.81, 23.52]	4.99	18.40	13.30	32.20

SD: Standard Deviation

is first available study data on vaginal absorption of oxytetracycline. Tetracyclines are distributed widely to tissues and body fluids (3). They also cross the placenta to reach the fetus and are excreted in breast milk (3). Oxytetracycline is considered teratogenic (22), however in doses larger than therapeutic ones (1). Absorption after oral administration is approximately 60-70% for oxytetracycline (3). Oral dosages of oxytetracycline of 500 mg every 6 hours produce peak blood levels of 4-6 mcg/mL (3). Intravenously injected tetracyclines gives somewhat higher levels, but only temporarily (3). In conclusion, concentration after vaginal administration of 100 mg oxytetracycline once daily was 250 times smaller than after oral dosages of oxytetracycline of 500 mg every 6 hours.

Strengths and limitations

The strength of this study is the detailed design as a prospective multicenter study. The study protocol was well-structured and included a set of statistical analyses. Investigators ensured the correctness of patient data, clinical examinations, and laboratory results. Regular quality controls were performed. Serum samples were uniformly collected, and assays carried out by skilled laboratory technicians in the Central Lab. Methods used were compliant with the highest standards. The study included well-defined population. The limitation of our study is that the study was open label. A randomized controlled trial would have provided a higher level of evidence. However, there was no appropriate medication to compare within the study parameters. A minor limitation of this study was the single-country design.

CONCLUSION

Based on the analysis of all efficacy and safety outcomes, it can be concluded that the Geonistin® vaginal tablets have a beneficial effect on the pathology of "unspecific and mixed vulvovaginal infections characterized by vaginal discharge". The clinical cure rate in the ITT population was high at 81.5%, 95% CI (0.75-0.87), $P<0.001$, and the cure rate was 84.2%, 95% CI (0.78-0.89), $P<0.001$ in the PP population. Subjects not classified as clinically cured by the vaginal tablets were in the clinical improvement group with no subject failing the trial. Additionally, microbiological and Nugent score efficacy measures confirmed clinical effectiveness. Only 15 AE occurred in total during the study. However, none of them were serious. The beneficial efficacy results of the drug can be achieved with few and non-serious AE, of which only one (vulvovaginal discomfort) was related to the drug according to the investigator. Of the 15 individuals chosen for PK analysis, 4 (26.7%) had oxytetracycline values below 10 ng/mL and the remaining subjects had values between 13.3 and 32.2 ng/mL.

In conclusion, the present study has confirmed the effectiveness and safety of the Geonistin® vaginal tablet in patients with unspecific and mixed vulvovaginal infections characterized by vaginal discharge.

Ethical approval

The study received approval from the Central Ethics Committee (CEC) and written approval of Ministry of the health of the Republic of Croatia. The study was performed in accordance with the Declaration of Helsinki (23), in compliance with local regulations; according to PLIVA Croatia Ltd. standard procedures, this clinical study was registered on clinical trials registry websites (EudraCT number: 2016-000078-39). This clinical study was conducted in accordance with the International Conference on Harmonization (ICH) Good Clinical Practice (GCP) Consolidated Guideline (E6) and applicable national and local laws and regulations, the European Union (EU) Directive 2001/20/EC on the approximation of the laws, regulations, and administrative provisions, and the sponsor's Standard Operating Procedures (SOPs). Written informed consent was obtained from each patient before any study-specific procedures or assessments were done. Patients privacy was ensured by an identification code (i.e. identification number).

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Author contributions

Contributed significantly to the execution, and/or analysis and interpretation of data: VŠ, AKL, MG, MMK, IMB. Writing and revising the manuscript for intellectual content: VŠ, AKL, MG, MMK, IMB. Approved the manuscript for submission: VŠ, AKL, MG, MMK, IMB.

Conflict of interest

This study was sponsored by PLIVA Croatia Ltd., Zagreb, Croatia. AKL, MMK, IMB are employees of PLIVA Croatia LTD, MG is employee of Teva Pharmaceuticals Europe BV.

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