In Vitro Activity of Fenticonazole against *Candida* and Bacterial Vaginitis Isolates Determined by Mono- or Dual-Species Testing Assays

Maurizio Sanguinetti,^{a,b} Emilia Cantón,^c Riccardo Torelli,^a Fabio Tumietto,^d Ana Espinel-Ingroff,^e Brunella Posteraro^{f,g}

^aDipartimento di Scienze di Laboratorio e Infettivologiche, Fondazione Policlinico Universitario A. Gemelli IRCCS, Rome, Italy

^bIstituto di Microbiologia, Università Cattolica del Sacro Cuore, Rome, Italy

^cUnidad de Microbiología Experimental, Grupo Multidisciplinar de Infección Grave, Instituto de Investigación Sanitaria, Hospital La Fe, Valencia, Spain

^dUnità di Malattie Infettive, Dipartimento di Scienze Mediche e Chirurgiche, Ospedale S. Orsola-Malpighi, Università di Bologna, Bologna, Italy

^eVCU Medical Center, Richmond, Virginia, USA

^fDipartimento di Scienze Gastroenterologiche, Endocrino-Metaboliche e Nefro-Urologiche, Fondazione Policlinico Universitario A. Gemelli IRCCS, Rome, Italy

^gIstituto di Patologia Medica e Semeiotica Medica, Università Cattolica del Sacro Cuore, Rome, Italy

ABSTRACT We determined the *in vitro* activity of fenticonazole against 318 vaginitis isolates of *Candida* and bacterial species and selected 28 isolates for time-kill studies. At concentrations equal to 4× MIC, fenticonazole reached the 99.9% killing endpoint by ~10 h for *Staphylococcus aureus*, *Streptococcus agalactiae*, and *Escherichia coli* and by ~17 h for *Candida albicans* and *Candida parapsilosis*; and at concentrations equal to 8× MIC, by ~19 and ~20 h for *Candida glabrata* and *Candida tropicalis*, respectively. At concentrations equal to 2× MIC, fenticonazole required ~20 h to reach the above endpoint against *C. albicans* in mixed culture with *S. aureus*, *S. agalactiae*, or *E. coli* versus ~17 h against *C. albicans* in pure culture. Supra-MICs are achievable in topically treated patients' vaginal surfaces.

KEYWORDS antimicrobial susceptibility testing, fenticonazole, targeted therapy, vaginal isolates

Candida vulvovaginitis (here referred to as vaginitis) is a common condition affecting up to 75% of women at least once in their lifetime (1). *Candida albicans* is the most frequent cause of this nonfatal, yet distressing disease (2), although species such as *Candida glabrata*, *Candida parapsilosis*, and *Candida tropicalis* have recently been associated with vaginitis (3). Notably, azole-resistant *Candida* isolates may be responsible for recurring and refractory vaginal infections (4), which are debilitating conditions (5). Multiple factors are associated with symptomatic *Candida* infection, with symptoms likely determined by the yeast's overgrowth and penetration of vulvovaginal epithelial cells (1). While topical and oral antifungal azoles are equally effective (6), treatment of symptomatic *Candida* vaginitis with topical azoles may necessitate longer courses to eradicate the infection (7).

Despite occurring rarely (<5%), mixed vaginitis is due to the simultaneous presence of at least two vaginal pathogens (e.g., a bacterium and a *Candida* species) that require the use of dual (e.g., antibacterial and antifungal) therapy or possibly broad-spectrum monotherapy for complete eradication of concurrent symptoms (8). Although underestimated until recently (9), coinfecting Gram-positive and Gram-negative bacteria are important causes of aerobic vaginitis (10), with *Streptococcus* species (e.g., *Streptococcus agalactiae*), *Staphylococcus aureus*, and *Escherichia coli* being the most prevalent pathogens identified in symptomatic women (11). Note that *S. aureus* is strongly attached to the hyphae of *C. albicans*, so it is less susceptible to antibacterial treatment (12).

Citation Sanguinetti M, Cantón E, Torelli R, Tumietto F, Espinel-Ingroff A, Posteraro B. 2019. *In vitro* activity of fenticonazole against *Candida* and bacterial vaginitis isolates determined by mono- or dual-species testing assays. Antimicrob Agents Chemother 63:e02693-18. <https://doi.org/10.1128/AAC.02693-18>.

Copyright © 2019 American Society for Microbiology. All Rights Reserved.
Address correspondence to Maurizio Sanguinetti, maurizio.sanguinetti@unicatt.it.

Received 3 January 2019

Returned for modification 25 February 2019

Accepted 2 May 2019

Accepted manuscript posted online 6 May 2019

Published 24 June 2019

Accordingly, interaction of *C. albicans* with *S. aureus* (or various other bacterial species) may influence the antimicrobial susceptibility of both organisms when evaluated in *in vitro* assays. This should mimic the results of mixed cultures of bacterial species exposed to antibiotics (13, 14).

Among the available azoles for clinical use (see ref. 15 for historical review), fenticonazole is an antifungal imidazole derivative, which was synthesized and developed by Recordati S.p.A. (Milan, Italy) about 3 decades ago for the topical treatment of dermatomycoses and *Candida* vaginitis (16). Interestingly, whereas the gamut of activity of fenticonazole comprises bacteria commonly associated with symptomatic vaginal discharge, the intravaginal administration of fenticonazole has shown efficacy in patients with *Candida* vaginitis, bacterial vaginosis, or mixed infection (17). Therefore, fenticonazole may be a topical alternative to multiagent antimicrobial treatment of mixed bacterial and fungal infections (17).

The previous evaluation of fenticonazole MICs for single cultures of *C. albicans* and *C. glabrata* did not include time-kill assays using single or mixed (i.e., with bacteria) cultures of *Candida* species (18). In the present study, we first investigated the dynamics of fenticonazole-induced killing in monospecies testing assays for vaginal *Candida* and bacterial isolates. Then, we determined the fenticonazole killing activity for dual species using mixed cultures of *C. albicans* with *S. aureus*, *E. coli*, or *S. agalactiae*.

We obtained an anonymized series of microbial isolates from symptomatic women with vaginitis throughout 18 months from the clinical microbiology laboratory at the Fondazione Policlinico Universitario A. Gemelli IRCCS of Rome (Italy). At the collection time, the isolates were identified using matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) and stored at -80°C until testing. We serially diluted fenticonazole [1-(2,4-dichlorophenyl)-2-(*N*-imidazolyl) ethyl 4-phenylthiobenzyl ether nitrate; Recordati] from a $100\times$ stock solution in dimethyl sulfoxide (Merck KGaA, Darmstadt, Germany) to achieve a 0.008- to $128\text{-}\mu\text{g/ml}$ final concentration range. Antimicrobial susceptibility testing was performed according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) documents for testing yeasts (http://www.eucast.org/ast_of_fungi/) and bacteria (http://www.eucast.org/ast_of_bacteria/). Antifungal MICs were the minimum concentration that yielded $\geq 50\%$ growth reduction (http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/AFST/Antifungal_breakpoints_v_7.0.pdf). The testing media were either RPMI 1640 plus 2% glucose broth buffered with morpholinepropanesulfonic acid or unsupplemented cation-adjusted Mueller-Hinton (MH) broth. The control strains were *C. parapsilosis* ATCC 22019, *Candida krusei* ATCC 6258, *S. aureus* ATCC 25923, and *E. coli* ATCC 25922. The time-kill experiments were determined using inocula from exponential growth cultures obtained for each isolate starting from an overnight culture at 37°C in agitation. For pure-culture time-kill experiments, we used $100\text{ }\mu\text{l}$ of the exponentially growing cultures in 5 ml of prewarmed RPMI 1640 or MH medium with or without (control) an antifungal to reach an inoculum of 1×10^5 to 5×10^5 CFU/ml. For mixed-culture time-kill experiments, we inoculated $100\text{ }\mu\text{l}$ of each of two desired microbial species (1:1) into the same MH medium volume. The fenticonazole concentrations tested were equivalent to $0.5\times$, $1\times$, $2\times$, $4\times$, and $8\times$ the MIC value, as determined for each species. Control and each test solution vials were incubated at 37°C with agitation in an Eppendorf ThermoMixer C (Eppendorf AG, Hamburg, Germany). A total of $100\text{-}\mu\text{l}$ aliquots from control and test solution vials for microbial quantification were removed at 0, 2, 4, 6, 8, 12, and 24 h. The CFU per milliliter were obtained as 10-fold serial dilutions in phosphate-buffered saline and plated ($50\text{-}\mu\text{l}$ samples) onto Trypticase-soy (bacterial species) or Sabouraud (fungal species) agars. The plates were incubated at 37°C for 24 to 48 h. The lower limit of accurate and reproducible microbial quantitation was 20 CFU/ml. Each experiment was performed three times to increase statistical significance and evaluated the antifungal carryover effect using the method reported by Cantón et al. (19). According to previous studies (19–21), we analyzed and compared killing kinetics by fitting time-kill data to the exponential equation $N_t = N_0 \times e^{-Kt}$ (t , incubation time; N_t , viable cells at time t ;

TABLE 1 Susceptibility of 318 vaginal isolates to fenticonazole determined by the EUCAST broth microdilution method

Species ^a (no. of isolates)	MIC (μg/ml)		
	MIC ₉₀	MIC ₅₀	Range
<i>E. coli</i> (25)	0.125	0.03	0.016–0.25
<i>S. agalactiae</i> (30)	0.03	0.016	0.008–0.125
<i>S. aureus</i> (35)	1	0.5	0.25–4
<i>C. albicans</i> (51)	0.06	0.03	0.016–0.25
<i>C. glabrata</i> (44)	0.25	0.125	0.06–0.5
<i>C. tropicalis</i> (39)	0.125	0.06	0.03–0.25
<i>C. parapsilosis</i> (52)	0.03	0.016	0.008–0.125
<i>G. vaginalis</i> (42)	0.03	0.016	0.008–0.03

^aMueller-Hinton and RPMI 1640 broths were the media used for performing antimicrobial susceptibility testing of the listed species of bacteria and *Candida*, respectively, according to EUCAST recommendations.

N_o , starting inoculum; K , killing rate). The exponential equation transformed into a line by applying natural logarithms ($\log N_t = \log N_o + Kt$) assessed the goodness of fit of the data by the r^2 value (>0.8). The fungicidal activities were compared by the K values that indicated killing or growth. Thus, the K values required to calculate time in hours to reach 50% (t_{50} , $0.30103/K$), 90% (t_{90} , $1/K$), 99% (t_{99} , $2/K$), and 99.9% ($t_{99.9}$, $3/K$) reductions in growth were compared with those for the starting inoculum.

Table 1 shows the fenticonazole MIC results for the 318 vaginal isolates tested. MICs for *S. aureus* ($n = 35$) were slightly higher than those for the other species, including *E. coli* ($n = 25$), *S. agalactiae* ($n = 30$), *C. albicans* and other *Candida* species ($n = 186$, in

FIG 1 Fenticonazole time killing of *Candida* (*C. albicans*, *C. glabrata*, *C. parapsilosis*, and *C. tropicalis*) and bacterial (*S. aureus*, *S. agalactiae*, and *E. coli*) species in pure cultures. Error bars, means $\pm$ SD of independently determined values for four isolates of each indicated species. Broken lines, $\geq 99.9\%$ growth reduction compared with that of the initial inoculum (fungicidal effect).

TABLE 2 Mean times to achieve 50%, 90%, 99%, and 99.9% growth reductions from the initial inoculum at the indicated multiple of the MIC of fenticonazole

Isolate tested, by culture type	Mean time to achieve growth reduction (h) of ^a :											
	t_{50}			t_{90}			t_{99}			$t_{99.9}$		
	2× MIC	4× MIC	8× MIC	2× MIC	4× MIC	8× MIC	2× MIC	4× MIC	8× MIC	2× MIC	4× MIC	8× MIC
Pure culture												
<i>C. albicans</i>	1.74	1.70	1.93	5.78	5.65	6.43	11.56	11.31	12.85	17.34	16.95	19.28
<i>C. glabrata</i>	NA ^b	>48	1.87	NA	>48	6.20	NA	>48	12.39	NA	>48	18.59
<i>C. parapsilosis</i>	10.45	1.74	1.87	34.72	5.77	6.21	>48	11.53	12.41	>48	17.30	18.62
<i>C. tropicalis</i>	NA	NA	2.05	NA	NA	6.80	NA	NA	13.61	NA	NA	20.41
<i>S. aureus</i>	1.21	1.06	0.94	4.03	3.53	3.14	8.06	7.06	6.27	12.10	10.60	9.41
<i>S. agalactiae</i>	1.12	1.03	0.92	3.73	3.43	3.04	7.46	6.87	6.09	11.19	10.30	9.13
<i>E. coli</i>	1.12	1.03	0.94	3.72	3.44	3.13	7.43	6.87	6.26	11.15	10.31	9.40
Mixed culture												
<i>C. albicans</i>	2.08	1.57	1.86	6.93	5.22	6.18	13.87	10.45	12.36	20.81	15.68	18.54
+ <i>S. aureus</i>	1.16	1.02	0.86	3.86	3.40	2.87	7.71	6.80	5.74	11.57	10.19	8.62
<i>C. albicans</i>	2.03	1.87	1.77	6.74	6.20	5.89	13.48	12.39	11.79	20.22	18.58	17.68
+ <i>S. agalactiae</i>	— ^c	1.07	0.99	—	3.54	2.99	—	7.08	6.57	—	10.62	9.86
<i>C. albicans</i>	2.05	2.00	1.78	6.83	6.64	5.91	13.66	13.28	11.83	20.49	19.93	17.74
+ <i>E. coli</i>	1.16	1.02	0.86	3.86	3.40	2.87	7.71	6.80	5.74	11.57	10.19	8.62

^a t_{50} , t_{90} , t_{99} , and $t_{99.9}$, time to achieve 50%, 90%, 99%, and 99.9% growth reduction, respectively. MICs for isolates tested as pure cultures ranged from 0.03 to 0.25 $\mu\text{g/ml}$ for *Candida* isolates and 0.06 to 0.25 $\mu\text{g/ml}$ for bacterial isolates. The drug concentrations used in the mixed cultures were equivalent to multiples of the MIC obtained with the *C. albicans* pure culture (0.25 $\mu\text{g/ml}$). The MIC value was, respectively, equal to or higher than those of the *S. aureus* (MIC, 0.25 $\mu\text{g/ml}$), *E. coli* (MIC, 0.25 $\mu\text{g/ml}$), and *S. agalactiae* (MIC, 0.125 $\mu\text{g/ml}$) isolates tested together with the *C. albicans* isolate in separate mixed cultures.

^bNA, not achieved.

^c—, *S. agalactiae* isolate (MIC, 0.125 $\mu\text{g/ml}$) was not tested at 2× MIC (i.e., 0.5 $\mu\text{g/ml}$), which would correspond to an MIC value of 0.25 $\mu\text{g/ml}$ for that isolate.

total), and *Gardnerella vaginalis* ($n = 42$). Conversely, *S. agalactiae*, *C. parapsilosis*, and *G. vaginalis* were the most susceptible tested species (MIC₉₀, 0.03 $\mu\text{g/ml}$). As for *Candida* species, *C. albicans* ($n = 51$; MIC₉₀, 0.06 $\mu\text{g/ml}$) and *C. parapsilosis* ($n = 52$; MIC₉₀, 0.03 $\mu\text{g/ml}$) isolates had slightly lower MICs than *C. glabrata* ($n = 44$; MIC₉₀, 0.25 $\mu\text{g/ml}$) and *C. tropicalis* ($n = 39$; MIC₉₀, 0.125 $\mu\text{g/ml}$) isolates. For use in the monospecies killing assays, we randomly selected 28 isolates of *C. albicans*, *C. glabrata*, *C. parapsilosis*, *C. tropicalis*, *S. aureus*, *S. agalactiae*, and *E. coli* (4 isolates/species). Fenticonazole MICs ($\mu\text{g/ml}$) were 0.125 (2 isolates) and 0.25 (2 isolates) for *C. albicans*, 0.125 (1 isolate) and 0.25 (3 isolates) for *C. glabrata*, 0.03 (2 isolates) and 0.06 (2 isolates) for *C. parapsilosis*, 0.06 (1 isolate) and 0.125 (3 isolates) for *C. tropicalis*, 0.25 (2 isolates) and 0.5 (2 isolates) for *S. aureus*, 0.06 (2 isolates) and 0.125 (2 isolates) for *S. agalactiae*, and 0.06 (1 isolate), 0.125 (2 isolates), and 0.25 (1 isolate) for *E. coli*.

Figure 1 depicts the time-kill curves of each of the seven species (means and standard deviations) tested as pure cultures, and Table 2 provides time-killing results for the same *Candida* and bacterial species. At fenticonazole concentrations of equal to 4× MIC for all species, the number of CFU/ml decreased >3 log units (99.9% killing) by incubation of 16.95 and 17.30 h for *C. albicans* and *C. parapsilosis*, respectively, and of 10.60, 10.30, and 10.31 h for *S. aureus*, *S. agalactiae*, and *E. coli*, respectively (Table 2). Compared to results in *C. albicans* and *C. parapsilosis* isolates, fenticonazole achieved a ≥ 3 -log decrease (killing endpoint) with a concentration of equal to 8× MIC by 18.59 h for *C. glabrata* and 20.41 h for *C. tropicalis* (Table 2) isolates. Interestingly, the lowest fenticonazole concentration to achieve the killing endpoint was 2× MIC at 17.34 h only for *C. albicans* isolates (Table 2).

To determine interspecies effects in the presence of fenticonazole, time-kill curves were obtained by cocultivating *C. albicans* (MIC, 0.25 $\mu\text{g/ml}$) with *S. aureus* (MIC, 0.25 $\mu\text{g/ml}$), *S. agalactiae* (MIC, 0.125 $\mu\text{g/ml}$), or *E. coli* (MIC, 0.25 $\mu\text{g/ml}$) isolates. Before assessment of dual-species killing, we assessed that either the MIC or the growth of *C. albicans* isolates in the MH broth was the same as in the RPMI broth. As shown in Fig. 2, the fungicidal effect of fenticonazole in the three mixed cultures was similar to that for the pure cultures of *C. albicans* isolates (see Fig. 1). However, fenticonazole reached the fungicidal endpoint (99.9% killing) faster at 4× MIC in mixed cultures of *C. albicans*

FIG 2 Fenticonazole time killing of *C. albicans* plus *S. aureus* (A), *C. albicans* plus *S. agalactiae* (B), and *C. albicans* plus *E. coli* (C) isolates in mixed cultures. The curves represent fungal (not bacterial) killing, and, accordingly, indicated MICs are those of *C. albicans*. Error bars, means $\pm$ SD of independently determined values for two isolates (one plus one) of each indicated species pair. Broken lines, $\geq 99.9\%$ growth reduction compared with that of the initial inoculum (fungicidal effect).

and *S. aureus* isolates than in pure cultures (15.68 versus 16.95 h, respectively) (Table 2). At concentrations of $2\times$ MIC, fenticonazole required ~ 20 h to reach the fungicidal endpoint against mixed cultures of *C. albicans* and *S. aureus* (20.81 h), *S. agalactiae* (20.22 h), or *E. coli* (20.49 h) isolates. These findings suggest that those bacterial species may interfere with the activity of fenticonazole against *C. albicans* infection. Conversely, at $4\times$ MIC, fenticonazole reached the 99.9% bacterial killing endpoint at 10.19, 10.62, and 10.19 h for *S. aureus*, *S. agalactiae*, and *E. coli* isolates, respectively (Table 2). Because these values were the same as those for bacterial pure cultures, our data suggest that the presence of *C. albicans* isolates may hinder the bacterial protection by fenticonazole.

Figure 3 shows the effects of fenticonazole concentrations on the killing rates (K values) for *C. albicans*, *C. glabrata*, *C. parapsilosis*, and *C. tropicalis* infection alone or

FIG 3 Fenticonazole killing rates (K) for pure cultures of *C. albicans*, *S. aureus*, *S. agalactiae*, and *E. coli* (A) and for mixed cultures of *C. albicans* with *S. aureus*, *S. agalactiae*, or *E. coli* (B). In panel B, curves represent fungal (not bacterial) killing, and, accordingly, indicated MICs are those of *C. albicans*. Error bars, means $\pm$ SD of independently determined values for the isolates of each indicated species. Values above the broken lines indicate growth, and values below the broken lines indicate killing.

when the mixture included *C. albicans*, *S. aureus*, *S. agalactiae*, or *E. coli* isolates. Fenticonazole showed the maximum K value against *C. albicans* (-0.17 CFU/ml/h) at $2\times$ MIC, whereas we observed the highest values (-0.14 , -0.16 , and -0.14 CFU/ml/h, respectively) at $8\times$ MIC for *C. glabrata*, *C. parapsilosis*, and *C. tropicalis* isolates. The maximum K value for *C. albicans* plus *S. aureus* (-0.19 CFU/ml/h), *C. albicans* plus *S. agalactiae* (-0.16 CFU/ml/h), or *C. albicans* plus *E. coli* (-0.15 CFU/ml/h, respectively) was achieved at $4\times$ MIC for the fungal species.

In 2009, Antonopoulou et al. (18) found excellent agreement between EUCAST and CLSI methods when testing fenticonazole against *Candida* isolates (249 *C. albicans* and 11 *C. glabrata*) from vaginal infections. Similar to our data, EUCAST MIC₅₀ values ranged from 0.03 to 0.25 μ g/ml for *C. albicans* isolates and from 0.03 to 0.5 μ g/ml for *C. glabrata* isolates (18). Fenticonazole inhibits the *ERG11* product (the key enzyme in the biosynthetic pathway of ergosterol), leading to fungal cell membrane alterations; it also impairs the release of secreted aspartyl proteinase in *C. albicans* isolates and blocks the fungal cytochrome oxidases and peroxidases (15, 17). Additionally, fenticonazole completely inhibits *C. albicans* filamentation (i.e., hyphal formation) at the MIC or higher drug concentrations (15). As for antibacterial activity, we confirmed that the fenticonazole activity reported by Jones et al. (22) against organisms associated with bacterial vaginal (including the prototype *G. vaginalis*) and skin infection (i.e., *S. aureus* and streptococci). In that study, MIC₅₀/MIC₉₀ values were 1/2 μ g/ml for *S. aureus* (20 isolates) and $\leq 0.03/0.125$ μ g/ml for streptococci (24 isolates), and the MIC for 6 isolates was 0.125 μ g/ml for group B streptococci, including *S. agalactiae*. The antibacterial activity of fenticonazole relates to the selective production of a cytotoxic oxidative metabolite (17), which is similar to the induction of reactive oxygen species (ROS) in *C. albicans* by miconazole (23), which is also widely used for vulvovaginal candidiasis (7).

Aerobic vaginitis and candidiasis are common pathological conditions associated with vaginal discharge, ranking second and third after bacterial vaginosis. The empirical treatment of symptomatic women requires the use of an agent that has combined antibacterial and antifungal activities (16). In this context, comparative clinical studies showed that fenticonazole is more for topical than for other treatments. The azoles are usually fungistatic, but under certain conditions and formulations, some azoles, such as miconazole, can be fungicidal (23, 24). Like amphotericin and ciclopirox (different drug classes), miconazole induces a common oxidative-damage cellular death pathway that culminates in the formation of lethal ROS (25). ROS induction also underlies fenticonazole microbicidal activity as shown in our study, but it requires future investigation.

In conclusion, our MIC data reinforce the potent *in vitro* activity of fenticonazole against *Candida* and bacterial species, whereas time-kill data highlight its potential microbicidal at supra-MICs easily reached in topically treated patients for skin/mucosal surfaces. Additionally, we show that increasing fenticonazole concentrations allowed the potential interference between *C. albicans* and *S. aureus* or the other bacterial

species evaluated as mixed cultures to be overcome. Finally, we recommend the use of topical fenticonazole for the treatment of vaginal infections with different etiology, which may minimize the risk of selecting drug-resistant microbial strains.

ACKNOWLEDGMENTS

Recordati S.p.A. (Milan, Italy) provided fenticonazole nitrate salt (batch no. M0170/069) for *in vitro* testing assays.

M.S. received a research grant from Recordati to support the present study.

REFERENCES

- Sobel JD. 2007. Vulvovaginal candidosis. *Lancet* 369:1961–1971. [https://doi.org/10.1016/S0140-6736\(07\)60917-9](https://doi.org/10.1016/S0140-6736(07)60917-9).
- Donders GG, Sobel JD. 2017. *Candida* vulvovaginitis: a store with a butterfly and a show window. *Mycoses* 60:70–72. <https://doi.org/10.1111/myc.12572>.
- Gonçalves B, Ferreira C, Alves CT, Henriques M, Azeredo J, Silva S. 2016. Vulvovaginal candidiasis: epidemiology, microbiology and risk factors. *Crit Rev Microbiol* 42:905–927. <https://doi.org/10.3109/1040841X.2015.1091805>.
- Bhattacharya S, Sobel JD, White TC. 2016. A combination fluorescence assay demonstrates increased efflux pump activity as a resistance mechanism in azole-resistant vaginal *Candida albicans* isolates. *Antimicrob Agents Chemother* 60:5858–5866. <https://doi.org/10.1128/AAC.01252-16>.
- Denning DW, Kneale M, Sobel JD, Rautema-Richardson R. 2018. Global burden of recurrent vulvovaginal candidiasis: a systematic review. *Lancet Infect Dis* 18:e339–e347. [https://doi.org/10.1016/S1473-3099\(18\)30103-8](https://doi.org/10.1016/S1473-3099(18)30103-8).
- Sherrard J, Donders G, White D, Jensen JS, European IUSTI. 2011. European (IUSTI/WHO) guideline on the management of vaginal discharge, 2011. *Int J STD AIDS* 22:421–429. <https://doi.org/10.1258/ijsa.2011.011012>.
- van Schalkwyk J, Yudin MH, Infectious Disease Committee. 2015. Vulvovaginitis: screening for and management of trichomoniasis, vulvovaginal candidiasis, and bacterial vaginosis. *J Obstet Gynaecol Can* 37:266–274. [https://doi.org/10.1016/S1701-2163\(15\)30316-9](https://doi.org/10.1016/S1701-2163(15)30316-9).
- Sobel JD, Subramanian C, Foxman B, Fairfax M, Gygas SE. 2013. Mixed vaginitis—more than coinfection and with therapeutic implications. *Curr Infect Dis Rep* 15:104–108. <https://doi.org/10.1007/s11908-013-0325-5>.
- Tansarli GS, Skolidis T, Legakis NJ, Falagas ME. 2017. Abnormal vaginal flora in symptomatic non-pregnant and pregnant women in a Greek hospital: a prospective study. *Eur J Clin Microbiol Infect Dis* 36:227–232. <https://doi.org/10.1007/s10096-016-2787-5>.
- Sherrard J, Wilson J, Donders G, Mendling W, Jensen JS. 2018. 2018 European (IUSTI/WHO) International Union against sexually transmitted infections (IUSTI) World Health Organisation (WHO) guideline on the management of vaginal discharge. *Int J STD AIDS* 29:1258–1272. <https://doi.org/10.1177/0956462418785451>.
- Tansarli GS, Kostaras EK, Athanasiou S, Falagas ME. 2013. Prevalence and treatment of aerobic vaginitis among non-pregnant women: evaluation of the evidence for an underestimated clinical entity. *Eur J Clin Microbiol Infect Dis* 32:977–984. <https://doi.org/10.1007/s10096-013-1846-4>.
- Scheres N, Krom BP. 2016. *Staphylococcus-Candida* interaction models: antibiotic resistance testing and host interactions. *Methods Mol Biol* 1356:153–161. https://doi.org/10.1007/978-1-4939-3052-4_11.
- dos Santos KV, Diniz CG, Coutinho SC, Apolônio AC, de Sousa-Gaia LG, Nicoli JR, de Macêdo Farias L, de Carvalho MA. 2007. *In vitro* activity of piperacillin/tazobactam and ertapenem against *Bacteroides fragilis* and *Escherichia coli* in pure and mixed cultures. *J Med Microbiol* 56:798–802. <https://doi.org/10.1099/jmm.0.47112-0>.
- Riedel C, Reichl U. 2011. Interspecies effects in a ceftazidime-treated mixed culture of *Pseudomonas aeruginosa*, *Burkholderia cepacia* and *Staphylococcus aureus*: analysis at the single-species level. *J Antimicrob Chemother* 66:138–145. <https://doi.org/10.1093/jac/dkq394>.
- Fromtling RA. 1988. Overview of medically important antifungal azole derivatives. *Clin Microbiol Rev* 1:187–217. <https://doi.org/10.1128/CMR.1.2.187>.
- Veraldi S, Milani R. 2008. Topical fenticonazole in dermatology and gynaecology: current role in therapy. *Drugs* 68:2183–2194. <https://doi.org/10.2165/00003495-200868150-00007>.
- Tumietto F, Giacomelli L. 2017. Fenticonazole: an effective topical treatment for superficial mycoses as the first-step of antifungal stewardship program. *Eur Rev Med Pharmacol Sci* 21:2749–2756.
- Antonopoulou S, Aoun M, Alexopoulos EC, Baka S, Logothetis E, Kalam-bokas T, Zannos A, Papadias K, Grigoriou O, Kouskouni E, Velegraki A. 2009. Fenticonazole activity measured by the methods of the European Committee on Antimicrobial Susceptibility Testing and CLSI against 260 *Candida* vulvovaginitis isolates from two European regions and annotations on the prevalent genotypes. *Antimicrob Agents Chemother* 53:2181–2184. <https://doi.org/10.1128/AAC.01413-08>.
- Cantón E, Pemán J, Gobernado M, Viudes A, Espinel-Ingróff A. 2004. Patterns of amphotericin B killing kinetics against seven *Candida* species. *Antimicrob Agents Chemother* 48:2477–2482. <https://doi.org/10.1128/AAC.48.7.2477-2482.2004>.
- Cantón E, Espinel-Ingróff A, Pemán J, del Castillo L. 2010. *In vitro* fungicidal activities of echinocandins against *Candida metapsilosis*, *C. orthopsilosis*, and *C. parapsilosis* evaluated by time-kill studies. *Antimicrob Agents Chemother* 54:2194–2197. <https://doi.org/10.1128/AAC.01538-09>.
- Gil-Alonso S, Jauregizar N, Cantón E, Eraso E, Quindós G. 2015. *In vitro* fungicidal activities of anidulafungin, caspofungin, and micafungin against *Candida glabrata*, *Candida bracarensis*, and *Candida nivariensis* evaluated by time-kill studies. *Antimicrob Agents Chemother* 59:3615–3618. <https://doi.org/10.1128/AAC.04474-14>.
- Jones BM, Geary I, Lee ME, Duerden BI. 1989. Comparison of the *in vitro* activities of fenticonazole, other imidazoles, metronidazole, and tetracycline against organisms associated with bacterial vaginosis and skin infections. *Antimicrob Agents Chemother* 33:970–972. <https://doi.org/10.1128/AAC.33.6.970>.
- Thevissen K, Ayscough KR, Aerts AM, Du W, De Brucker K, Meert EM, Ausma J, Borgers M, Cammue BP, François IE. 2007. Miconazole induces changes in actin cytoskeleton prior to reactive oxygen species induction in yeast. *J Biol Chem* 282:21592–21597. <https://doi.org/10.1074/jbc.M608505200>.
- Vandenbosch D, Braeckmans K, Nelis HJ, Coenye T. 2010. Fungicidal activity of miconazole against *Candida* spp. biofilms. *J Antimicrob Chemother* 65:694–700. <https://doi.org/10.1093/jac/dkq019>.
- Belenky P, Camacho D, Collins JJ. 2013. Fungicidal drugs induce a common oxidative-damage cellular death pathway. *Cell Rep* 3:350–358. <https://doi.org/10.1016/j.celrep.2012.12.021>.