**Objectives:**Olorofim, an innovative antifungal from the orotomide family, inhibits dihydroorotate dehydrogenase, thereby disrupting pyrimidine synthesis. It shows potent fungicidal activity against filamentous fungi and is in late clinical development. Reliable epidemiological cutoff values require susceptibility data from diverse regions; however, data on Latin America are scarce. This study aimed to evaluate *in vitro* activity of olorofim against 14 *Aspergillus* species isolated from Argentinian patients, including cryptic and azole-resistant strains, and to compare its efficacy with seven antifungals from other classes.

**Methods:**We studied 62 contemporary clinical strains of 14 different species of *Aspergillus* spp., comprising: *A. fumigatus sensu stricto* (*s.s*) (n=24, including ten *Cyp51A* mutant), *A. terreus s.s.* (12), *A. niger s.s.* (9), *A. flavus s.s.* (6), and *A. parasitucus* (n=2). Additionally, one strain each of *A. nidulans*, *A. udagawe*, *A. lentulus*, *A. pseudoterreus*, *A. luchensis*, *A. brasiliensis*, *A. ustus*, *A. calidoustus,* and *A. oryzae* were included. Species identification was performed using phenotypic methods and confirmed through MALDI-TOF, as well as calmodulin and beta-tubulin gene sequencing. Susceptibility testing was performed following the CLSI M38 (3rd Ed.). and EUCAST 9.4 documents. Tested antifungal agents included olorofim, amphotericin B, anidulafungin, caspofungin, isavuconazole, itraconazole, posaconazole, and voriconazole. *A. flavus* ATCC204304 and *A. fumigatus* ATCC204305 were used as quality control strains. Geometric mean (GM) MIC values and ranges were determined to analyze results.

**Results:** The overall essential agreement (±2 log2 dilutions) between the CLSI and EUCAST methods for olorofim susceptibility testing was 96.03%. As EUCAST data did not show significant differences from CLSI, GM MIC data obtained using CLSI are presented to simplify interpretation. Among the antifungals tested, olorofim proved to be the most potent, with the lowest MIC values (0.0243 µg/mL and 0.0189 mg/L for CLSI and EUCAST, respectively). Olorofim activity was comparable to that of echinocandins (anidulafungin and caspofungin GM MICs 0.015 µg/mL and 0.037 µg/mL, respectively) and it displayed a potency nine- to >60-fold higher than azoles (isavuconazole, itraconazole, posaconazole and voriconazole GM MICs 1.56, 0.627, 0.223, and 0.609 µg/mL, respectively). For *A. fumigatus s.s.*, *A. terreus* s.s., *A. flavus* s.s., and *A. niger* s.s. all tested strains exhibited remarkably low olorofim MIC values (GM MICs 0.018, 0.006, 0.007, 0.07 µg/mL, respectively). Less common species, such as *A. parasiticus, A. ustus,* and *A. calidoustus*, showed higher olorofim MICs values, ranging from 0.125 to 1 µg/mL. Cryptic species within *Aspergillus* section *Fumigati* section, as well as *A. fumigatus s.s.* strains harboring *Cyp51A* substitutions associated with azole resistance, showed consistently low olorofim MIC values, all between 0.008 and 0.03 µg/mL.

**Conclusions:** Olorofim demonstrates exceptional activity against this collection of *Aspergillus* spp. This orotomide inhibits fungal growth at concentrations significantly lower than voriconazole and other azoles. While its GM values are comparable to echinocandins, these are not the preferred choice for aspergillosis treatment. Rare strains like *A. calidoustus* and *A. ustus* exhibited slightly elevated MICs. Olorofim's novel mechanism of action offers a promising option for managing azole-resistant strains, cryptic species, multidrug resistant-species, and polyene-resistant species, such as *A. terreus*.