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The incidence of *Candida* infections has been increasing, and although *C. albicans* is still the most prevalent species, an epidemiological shift of these pathogens to non *Candida albicans* *Candida* species has been observed. Since these *Candida* species present variable levels of susceptibility to antifungal agents, there is an increasing need for rapid and precise methods for susceptibility testing, such as the E-test method. To assure the correct performance of these tests, and the accuracy of the results, it is extremely important to perform quality control assays using reference strains, which have already the minimum inhibitory concentration (MIC) values established. Thus, the aim of this work, performed in the Service of Clinical Pathology of Hospital de São Marcos (Braga, Portugal), was to compare the values of MIC obtained with the E-test for reference strains with the literature ones. Since the E-test protocol does not involve a precise control of the inoculum volume, which could affect susceptibility results, it was also a goal of this study to assess the influence of the inoculum volume in the E-test readings. The susceptibility of ATCC strains *C. albicans* 90028, *C. parapsilosis* 22019 and *C. krusei* 6258 to the antifungal agents (Amphotericin B, Flucytosin, Caspofungin, Fluconazole, Itraconazole, Voriconazole, Ketoconazole and Posaconazole) was assessed by E-test. MIC determinations were obtained from the average of 20 assays performed in the same day. Moreover, E-test was performed with four different volumes (50, 150, 300 and 1500 μ L) of inoculum on 150 mm agar plates. MICs obtained for the ATCC strains were generally lower than those established by NCCLS for all antifungal agents, except for Caspofungin, whose MICs were similar to the established. Thus, the results highlight the idea that MIC values should be revised. Considering the influence of the inoculum volume in E-test method, significant differences were mainly detected between the most divergent volumes (50 and 1500 μ L).

Furthermore, E-test results using the normal procedure (swab) were more similar to the MICs obtained with the lower volumes. Therefore, this work indicates that MIC values obtained by the E-test method for the reference strains assayed is lower than the established by NCCLS and that the inoculum volume does not influence E-test MIC readings, as long as it is not too high.