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Activity of anidulafungin against Candida albicans isolated from clinical specimens

Aktywność anidulafunginy w stosunku do szczepów *Candida albicans* izolowanych z materiałów klinicznych

Yeasts belonging to *Candida* spp. are a part of the normal human microflora of mucocutaneus areas, mainly within the upper airways, gastrointestinal tract and vagina. *Candida albicans* is still the major aetiological agent of both community-acquired and nosocomial candidiases. Such infections are usually endogenous in origin [4, 9, 10]. Infections caused by *C. albicans* are an important medical problem in patients from several risk groups, including those undergoing surgical procedures, with intravenous drug administration, organ transplant recipients, oncology patients, with some endocrynological disorders (e.g. diabetes). Although *C. albicans* is usually sensitive to several available antifungals, e.g. azoles or amphotericin B, increasing drug resistance of pathogenic yeasts may contribute to therapeutic failures [7, 8]. Anidulafungin belongs to a unique class of new antifungals known as echinocandins. This agent kills yeast cells by inhibiting the biosynthesis of 1,3-β-D-glucan, an essential polysaccharide being a main structural component of the fungal cell wall, which provides structural cell integrity and osmotic stability. Anidulafungin has proven to be very effective against different clinically important yeasts, especially *Candida* spp. [2, 3, 5, 6, 8, 12]. However, there is a need to monitor sensitivity of *Candida* spp. clinical isolates to echinocandins, including anidulafungin, in order to assess the rate of resistance to these drugs [3, 6].

The aim of this paper was to analyse by the E-test procedure the *in vitro* activity of anidulafungin against 41 strains of *Candida albicans* isolated from different clinical specimens.

MATERIAL AND METHODS

Clinical specimens (e.g. blood, spit, urine, feces and swabs from oral cavity, throat and nose, ear, vagina or cervix) were obtained from the hospitalized patients. The specimens were immediately streaked onto CHROMagar Candida Medium (Becton Dickinson). The isolates were identified by biochemical microtest API 20 C AUX (bioMerieux) on the basis of assimilation of various substrates such as D-glucose, glycerol, calcium 2-keto-gluconate, L-arabinose, D-xylose, adonitol, xylitol, D-galactose, inositol, D-sorbitol, methyl-αD-glucopyranoside, N-acetyl-glucosamine, D-cellobiose, D-lactose, D-maltose, D-saccharose, D-trehalose, D-melezitose and D-rafinose.

Anidulafungin susceptibility was assessed by the E-test procedure (AB BIODISK) using RPMI 1640 medium (SIGMA-ALDRICH) buffered to a pH 7.0 with 0.165 M morpholine propanesulphonic acid (MOPS). The E-test is a quantitative technique for determining the minimum inhibitory concentration (MIC) of antimicrobial agents. MIC is the lowest concentration of antimicrobial agent that will inhibit the visible growth of microorganisms. Using E-test procedure, MIC is read directly from the scale in terms of mg/l at the point where the edge of the ellipse inhibition zone intersects the strip (Fig. 1). The MIC of anidulafungin for the reference yeast strain *C. parapsilosis* ATCC 22019 was 3 mg/l, i.e. within the recommended MIC range 0.5–4 mg/l.

RESULTS

All clinical isolates of *C. albicans* were susceptible to anidulafungin with MICs ranging from 0.003 to 0.012 mg/l. According to CLSI (Clinical Laboratory Standard Institute) the breakpoint of anidulafungin for susceptible strains of *C. albicans* is ≤ 2 mg/l.

Most of the assayed clinical *C. albicans* isolates – 20 (49%) were inhibited by anidulafungin with MIC = 0.006 mg/l (Fig. 2). The MIC_{50} and the MIC_{90} values for anidulafungin were calculated, defined as the MIC inhibited 50% or 90% of the isolates, respectively; these values were 0.004 mg/l or 0.006 mg/l, respectively.

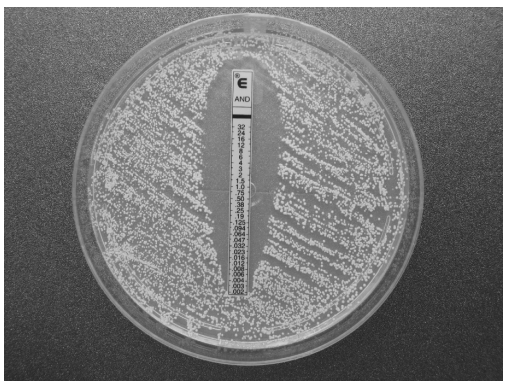

Fig. 1. Determination of sensitivity to anidulafungin of *C. albicans* clinical isolate by the E-test procedure

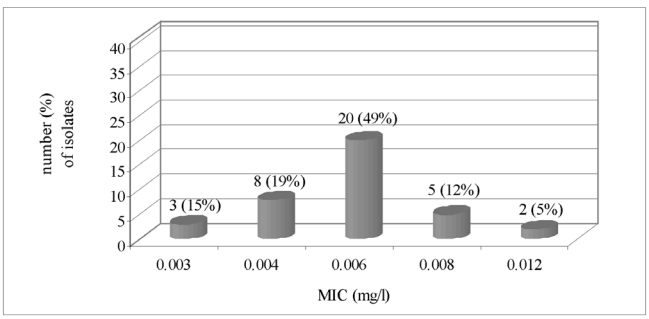

Fig. 2. The MICs of anidulafungin for *C. albicans* clinical isolates determined by the E-test procedure

DISCUSSION

The increased frequency of fungal infections in recent years is associated with several factors, including inappropriate use of antifungal drugs. This is a major cause for the emergence of resistant or multi-drug resistant strains, which may lead to many therapeutic failures. Therefore, determination of drug sensitivity of the isolated fungal species should be the basis of a rational, successful therapy [2, 4, 7, 8, 10, 12].

Anidulafungin has been proven to be highly active against different clinically important yeasts. This antibiotic shows a potent fungicidal activity *in vitro* against a broad range of *Candida* spp., including *C. albicans*. This agent is also effective *in vitro* against yeast strains resistant to amphotericin B and azoles, e.g. fluconazole [13]. Anidulafungin is recommended in treating candidiasis ranging from superficial infections, such as oral thrush and vaginitis, to systemic and potentially life-threatening diseases, e.g. esophageal candidiasis or candidemia [7, 9, 10, 13].

Our data indicate that all strains of *C. albicans* isolated from various clinical specimens were highly susceptible to anidulafungin (MIC $\leq$ 0.012 mg/l) as evaluated by the E-test procedure. According to other authors [2, 9, 11, 12, 13], the MIC values of anidulafungin for *C. albicans* determined by the broth microdilution method, recommended by CLSI as the reference method, were similar, ranging from 0.007 mg/l to 2 mg/l. Our data suggest that the E-test procedure may be used routinely for determination of sensitivity of *Candida* spp. to anidulafungin.

As found by other authors [7], MIC of anidulafungin for only a few clinical isolates of *C. albicans* were higher than 2 mg/l – 8 or 16 mg/l, indicating insensitivity of the isolates. It should be noted that echinocandins are a relatively new group of antifungals and resistance is rare, while the mechanism of resistance is still unknown [13].

The data presented in this paper showing the high *in vitro* activity of anidulafungin against *C. albicans* clinical isolates and those from literature [7, 8, 12] concerning *in vitro* data and *in vivo* data from animal models or from clinical trials, point to the clinical significance of anidulafungin as an alternative option in the therapy of candidiases, especially invasive ones.

CONCLUSIONS

Our data indicate that anidulafungin has potent *in vitro* activity against *C. albicans* strains isolated from various clinical specimens. Moreover, the E-test method seems to be a reliable alternative to the reference broth microdilution method and may provide another choice for clinical laboratories.

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SUMMARY

Infections caused by *C. albicans* are an important medical problem in people from risk groups, e.g. hospitalized patients. The aim of this paper was to analyse by the E-test procedure, the *in vitro* activity of anidulafungin against clinical isolates of *C. albicans.* This antifungal agent belongs to new class of echinocandins. The data presented in this paper indicate that all isolates of *C. albicans* from various clinical specimens were susceptible to anidulafungin. Our data and those from the literature confirm the validity of including anidulafungin for the list of drugs effective in the therapy of infections caused by *C. albicans*. Morever, the E-test procedure may be used routinely for determination of sensitivity of *Candida* spp. to anidulafungin.

STRESZCZENIE

Infekcje wywoływane przez *C. albicans* są ważnym problemem medycznym u osób z grup ryzyka, głównie pacjentów szpitali. Celem pracy była ocena wrażliwości klinicznych szczepów *C. albicans* na anidulafunginę metodą E-testów. Lek ten jest przedstawicielem nowej grupy antybiotyków przeciwgrzybiczych – echinokandin. Uzyskane dane świadczą o tym, że wszystkie szczepy *C. albicans* izolowane z materiałów klinicznych były wrażliwe na anidulafunginę. Nasze dane oraz dane literatury potwierdzają zasadność wpisania anidulafunginy na listę leków skutecznych w zakażeniach wywołanych przez drożdżaki, w tym *C. albicans*. Ponadto metoda E-testów może być stosowana w rutynowym oznaczaniu wrażliwości *Candida spp*. na anidulafunginę.