First report on echinocandin resistant Polish Candida isolates

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Purpose: Candida spp. are ranked as one of the four major causative agents of fungal infections. The number of infections caused by Candida species resistant to fluconazole, which is applied as the first line drug in candidiasis treatment, increases every year. In such cases the application of echinocandin is necessary. Echinocandin susceptibility testing has become a routine laboratory practice in many countries due to the increasing frequency of clinical failures during treatment with these drugs. Methods: We performed anidulafungin, micafungin and caspofungin susceptibility testing according to the microdilution broth method on 240 Candida isolates collected in Polish hospitals. Results: We identified 12 isolates resistant to all echinocandins within 240 examined isolates. Moreover, 6 of the examined samples were identified as rare Candida species and among them we observed very high echinocandin MIC values. Conclusion: Our research proves that in Poland there is a problem of echinocandin resistance. Moreover, we identified two species of Candida which are rare causative agents of human infections, and there was no reported incidence of such infections in Poland until now.

Key words: Candida infections, echinocandin resistance, minimal inhibitory concentration, C. palmiololephila, C. inconspicua

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Abbreviations: AND, anidulafungin; C., Candida; CLSI, Clinical and Laboratory Standards Institute procedure; CSP, caspofungin, GRASO, CHROMMagar Candida; ITS, Internal Transcribed Spacer; MCF, micafungin; MIC, Minimal Inhibitory Concentration

INTRODUCTION

Candida spp. are ranked as the fourth leading causative agent of fungal infections in intensive care units (Sanguinetti et al., 2015). About 90% of these infections are caused by Candida (C.) albicans, C. glabrata, C. parapsilosis, C. tropicalis, and C. krusei (Sanguinetti et al., 2015). So far, the most prevalent pathogen during candidaemia that was isolated has been C. albicans. According to the clinical practice guidelines, fluconazole and echinocandin are the first line drugs in empiric therapy in case of Candida infections (Pappas et al., 2015). The echinocandin group consists of three compounds: anidulafungin (AND), caspofungin (CSP) and micafungin (MCF). The choice of the appropriate antimycotics is related to the patient’s condition, as well as the type of infection. However, an increase in the number of fungal infections caused by non-albicans species, such as C. glabrata or C. krusei, showing natural resistance to fluconazole (Choi et al., 2009), is the reason for the application of echinocandins. Infections caused by C. glabrata are now the second most common cause of candidaemia in North America and Europe (Pappas et al., 2015), and result in increased mortality rates in patients with candidaemia (Cornely et al., 2014). The frequency of echinocandin resistance among Candida spp. differs depending on the species, the region of infection and the patient (Grossman et al., 2014). Studies conducted in different countries have shown a variety of C. albicans resistant to echinocandin. According to Castanheira et al.’s research, echinocandin resistance among C. albicans is at approximately 3% (Castanheira et al., 2010). However, echinocandin resistance among C. glabrata seems to be a serious problem. Studies conducted from 2001 to 2010 had shown an increase in resistance from 2-3% to more than 13% among the C. glabrata strains (Perlin, 2015).

A report from 2015 made in Italy in accordance with the Clinical and Laboratory Standards Institute procedure (CLSI) has shown the resistance to AND (2.7%), CSP (16.2%) and MCF (13.5%) among C. glabrata isolates (Montagna et al., 2015). So far, there has been no information about clinical isolates being resistant to echinocandin in Poland. The frequency of non-albicans infections in Poland is increasing. The mortality of patients with candidiasis was 8.5%, in 118 clinical cases of infections in Polish hospitals (Dzierzanowska-Fangrat et al., 2014). Research conducted in 2013 at 20 Polish hospitals based on a two years period, reported 302 cases of candidaemia. The highest number of infections was found in intensive care (30.8%) and surgical (29.5%) units, whereas hematological units reached 15.9%, and the lowest number of infections was seen in neonatal units (4.6%). The most frequent isolated species was C. albicans (50.9%). The frequency of C. krusei and C. tropicalis was at 24% and 18%, respectively, in the hematological units. The distribution of C. glabrata and C. parapsilosis was at 14.1% and 13.1%, and there was no statistically significant differences between the departments (Nawrot et al., 2013). The results, published in 2008, 2012, 2014 and 2017, had shown according to the results of E-tests there were no any non-Candida isolates resistant to caspofungin and micafungin (Szymankiewicz & Dancewicz, 2008; Wieczorek et al., 2008; Kurnatowska et al., 2012; Golaś et al., 2014; Sulik-Tyszka et al., 2017).

MATERIALS

In this study we identified and examined AND, CSP and MCF susceptibility of 240 Candida isolates, collected in four Polish hospitals in Gdańsk, Szczecin, Warsaw and Wrocław, between the years of 2008 to 2012. The isolates originated from a variety of clinical specimens, for example isolated from swabs of the mouth, throat, faeces, urine, blood, and bronchopulmonary lavage fluid.
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METHODS

All isolates were cultured on CHROMagar Candida (GRASO) medium and incubated for 48 h at 35°C. For the species identification, ITS1, 5.8S RNA, ITS4 (White et al., 1990) regions was amplified and then sequenced. DNA extractions were performed according to an earlier described procedure (Brillowska-Dąbrowska et al., 2013). 2x Master Mix HighGC (A&A Biotechnology) was applied for all of the PCR assays performed. PCR products were purified (Clean-up, A&A Biotechnology) and sequenced (Macrogen).

Table 1. In vitro echinocandin susceptibility test results of 240 isolates of Candida spp.

| MIC breakpoint [mg/l] | 137 isolates of C. albicans | 72 isolates of C. glabrata | 17 isolates of C. krusei | 8 isolates of C. parapsilosis | 6 other isolates (5 C. palmioleophila and 1 C. inconspicua) |
|----------------------|-------------------------------|---------------------------|------------------------|-----------------------------|-------------------------------------------------|
| S I R ≤0.008 0.016 0.031 0.063 0.125 0.25 0.5 1 2 ≥4 | S I R ≤0.008 0.016 0.031 0.063 0.125 0.25 0.5 1 2 ≥4 | S I R ≤0.008 0.016 0.031 0.063 0.125 0.25 0.5 1 2 ≥4 | S I R ≤0.008 0.016 0.031 0.063 0.125 0.25 0.5 1 2 ≥4 | S I R ≤0.008 0.016 0.031 0.063 0.125 0.25 0.5 1 2 ≥4 |
| AND ≤0.25 0.25 ≥1 79 23 14 11 4 1 2 3 – – | AND ≤0.12 0.25 ≥0.5 3 10 32 13 5 – 4 4 1 – | AND ≤0.25 0.25 ≥1 – 2 3 11 – – – – 1 | AND ≤0.25 0.5 ≥8 – – – – 1 – – – – 1 | Lack of MIC breakpoint |
| MCF ≤0.25 0.5 ≥1 28 69 22 9 3 – 3 3 – – | MCF ≤0.12 0.25 ≥0.5 7 31 19 3 3 1 – 7 1 – | MCF ≤0.25 0.5 ≥1 – – – – 12 3 – – – – 1 | MCF ≤0.25 0.5 ≥8 – – – – 1 – – – – 1 | |
| CSP ≤0.25 0.5 ≥1 2 24 34 28 33 7 3 5 – 1 | CSP ≤0.06 0.12 ≥0.25 – 2 7 22 22 10 2 5 – 2 | CSP ≤0.25 0.5 ≥1 – – – – 1 2 13 – 1 | CSP ≤0.25 0.5 ≥8 – – – – 1 – – – – 1 | |
| | | | | | 6 other isolates (5 C. palmioleophila and 1 C. inconspicua) |
| | | | | | 0.008 0.016 0.031 0.063 0.125 0.25 0.5 1 2 ≥4 |
| | | | | | 2 – – – – – 2 – – – 2 |
| | | | | | 2 – – – – – 1 1 – 2 |
| | | | | | – 2 – – – – 1 – 3 |
Sequence analysis was performed with VectorNTI (Informax).

Minimal Inhibitory Concentrations (MIC) were determined by broth microdilution and the results were read visually following 24 h incubation, as the lowest concentration of the drug that caused a complete growth inhibition. Also, Candida albicans ATCC 90028 and Candida krusei ATCC 6258 strains were used as controls. All tests were performed in triplicates and in case of discrepancies they were repeated. AND (Pfizer), CSP (Sigma-Aldrich), MCF (Astellas) were obtained as a standard powder.

RESULTS

Among 240 Candida samples, by sequencing an rRNA fragment we identified: 137 C. albicans, 72 C. glabrata 17 C. krusei, 8 C. parapsilosis and 6 strains belonging to two rare Candida species: 5 C. palmioleophila and 1 C. inconspicua strain. CHROMagar Candida correctly identified 93.4% C. albicans, 97.2% C. glabrata, 80% C. krusei strains. C. palmioleophila developed a turquoise color on CHROMagar, while C. inconspicua colonies were pink to violet.

Results of three echinocandins susceptibility examination tests are presented in Table 1. Among 137 C. albicans isolates, as many as 3 had shown a significant decrease in susceptibility to AND, 6 to CSP and 3 to MCF (minimal inhibitory concentration value for all echinocandins ≥1 mg/L); 2 isolates were immediately resistant to AND, 3 to CSP, and 3 to MCF. In general, only 3/137 (2.2%) isolates of C. albicans were resistant to all echinocandins.

Out of 72 C. glabrata isolates, as many as 9 had shown a significant decrease in susceptibility to AND, 19 to CSP and 8 to MCF (MIC values: ≥0.5 mg/L, ≥0.5 mg/L, ≥0.25 mg/L, respectively). Only 1 isolate was immediately resistant to MCF and 22 to CSP, (MIC value ≥0.125 mg/L, ≥0.25 mg/L). Only 7 isolates were resistant to all three echinocandins.

In the case of C. krusei we observed a decrease in CSP susceptibility of 14/17 isolates. However, these isolates were sensitive to AND and MCF. According to the echinocandin mechanism of action and well known technical problems with establishing MIC for CSP, it is unlikely that such a large percentage of isolates would show resistance only to one antibiotic from this group. Thus, none of the C. krusei isolates were probably not resistant to echinocandins because they were neither resistant to AND nor MCF. We identified only 1 isolate which was resistant to three echinocandins (MIC values ≥4 mg/L for all echinocandins).

Among 8 C. parapsilosis we identified one resistant isolate to all echinocandins (MIC values ≥8 mg/L).

The MIC values of rare species of Candida were very high, but there is no echinocandin breakpoint established for these species (probably due to the low frequency of occurrence). The MIC value ≥4 was observed for one isolate of C. palmioleophila, and the same MIC value for the three echinocandins is exhibited by C. inconspicua. Two isolates of C. palmioleophila had MIC values ≤0.016 mg/L. The two isolates had a different MIC value depending on the examined antifungics. The results of echinocandin susceptibility testing of these rare Candida isolates are listed in Table 2.

DISCUSSION

Epidemiological studies on Candida infections are conducted in many countries (Choi et al., 2009). Various data are available on the prevalence of resistance to echinocandins among fungi of the Candida genus. These studies report that the occurrence of resistant isolates varies depending on the site of infection and the patient population. Previous epidemiological studies on resistance of Candida spp. in Poland are an insufficient source of data. There are two reports (Szmyankiewicz & Dancewicz, 2008; Wieczorek et al., 2008) from 2008 on caspofungin susceptibility testing performed with E-tests on isolates collected in the Polish hospitals. All of the 29 and 93 examined Candida isolates were susceptible to echinocandins. Another two reports from 2012 and 2014 had shown that there were no resistant Candida isolates within the 10 and 150 specimens collected in the Polish hospitals (Kurnatowska et al., 2012; Golaś et al., 2014). The latest echinocandin susceptibility testing was performed with E-tests in 2017. Only 46 isolates were examined and echinocandin resistance was not found (Sulik-Tyszka et al., 2017).

Our research has shown that the echinocandin resistance of Candida isolates is a problem in Poland, especially within non-albicans species — 9.7% C. glabrata isolates were echinocandins resistant (7/72). Echinocandins susceptibility testing had shown that out of all the 240 isolates of Candida spp., 14 (5.8%) were resistant to AND; 40 (16.6%) to CSP, and 13 (5.4%) to MCF.

What is very interesting, we isolated 6 isolates belonging to two species that are rarely identified as a cause of human infections. C. inconspicua is described in the
literature as a fluconazole-resistant and amphotericin B susceptible and is isolated from immunocompromised patients (Baily et al., 1997; Sugita et al., 2004; Guitard et al., 2013; Majoros et al., 2005). We identified one isolate of C. intractuica which was characterized by very high echinocandins MIC.

Out of 5 C. palmioleophila isolates, 3 were characterized by high echinocandins MIC value. According to a variety of data, C. palmioleophila could be resistant to fluconazole and susceptible to other antifungotics, e.g. echinocandins (Lau et al., 2017; Meletadis et al., 2016), but there is also some information about elevated caspofungin MIC of C. palmioleophila (Brilhante et al., 2017). C. palmioleophila were found in animal mornifrons (Sokól et al., 2018) and there are only a few data available on C. palmioleophila as an etiological agent of human infections (Trousèv et al., 2017).

It should be emphasized that data on previous echinocandins exposure (type and duration of antifungal therapy of patients) of the isolates examined in our study are not available. However, this does not change the fact that we indicate the problem of echinocandin resistance in Poland. Moreover, as the number of infections caused by Candida species resistant to fluconazole which is applied as the first line drug in candidiasis treatment in Poland increases, the occurrence of echinocandins resistance among Candida isolates should be examined.

Declaration of interest
The authors report no conflicts of interest.

Ethics approval
This study was exempt for ethics board approval as patient-specific public health information was not collected.

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