ANTIFUNGAL SUSCEPTIBILITY TESTING OF CANDIDA SPECIES ISOLATED FROM BLOOD STREAM INFECTION BY USE OF VITEK 2 SYSTEM

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ABSTRACT

BACKGROUND

Candida blood stream infections have increased drastically by 2 to 5 folds in a tertiary care hospital over the past decade. In spite of advances in diagnosis and treatment, candidaemia is one of the major cause of morbidity and mortality in healthcare facility. The changing antifungal spectrum of Candida blood stream infection has generated great concern about the emergence of drug resistance strains of azole and its clinical outcome.

The aim of the study is to speciate and determine the antifungal susceptibility testing of candida species isolated from bloodstream infection by use of VITEK 2 system.

MATERIALS AND METHODS

In a prospective study, a total of 50 Candida species isolated from bloodstream infection were subjected to identification and antifungal susceptibility testing by VITEK 2 automated system.

RESULTS

Among the 50 Candida blood isolates, C. tropicalis was the predominant strain isolated in 22 (44%) isolates, followed by C. albicans in 15 (30%), C. glabrata in 7 (14%), C. krusei in 3 (6%), C. parapsilosis in 2 (4%) and C. kefyr in 1 (2%) isolate. All the C. albicans showed 100% susceptibility to fluconazole, voriconazole, flucytosine and amphotericin B. C. glabrata showed 100% resistance to azoles. C. krusei showed 100% resistance to fluconazole. A 4.5% C. tropicalis showed resistance to amphotericin B.

CONCLUSION

The successful treatment of Candida infections in blood depends on the rapid identification of the species and sensitivity patterns to antifungal agents. VITEK 2 system is a valuable tool for identification and AST as it is rapid and less cumbersome. Amphotericin B and voriconazole seem to be suitable drugs for empirical therapy in severe cases and fluconazole is not suitable because most of the Candida species are resistance to them.

KEYWORDS

Candida Species, Antifungal Agents, Antifungal Resistance.

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BACKGROUND

Candida blood stream infections have increased drastically by 2 to 5 folds in a tertiary care hospital over the past decade. Candida species is the fourth most common cause of blood stream infection. In spite of advances in diagnosis and treatment, candidaemia is one of the major cause of morbidity and mortality in healthcare facility. The incidence of nosocomial candidaemia is on the rise due to immunocompromised host status and surgical intervention.[1,2]

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Importance of Candida species in nursery and intensive care setup is increasingly being recognised. Candida species accounts for 9 to 13% of all blood stream isolates in neonatal intensive care unit.[3] Higher incidence of fungal infections in hospitalised patients has resulted in the use of antifungal agents, especially fluconazole, which remains a first line antifungal drug of choice. Even fluconazole-resistant species have gained importance and Candida albicans strains with decreased susceptibility to fluconazole are increasing been reported.[4] Increased prevalence of non-albicans Candida is of great importance because of its decreased susceptibility to antifungal agents. Fluconazole and itraconazole resistances are observed in C. tropicalis and C. parapsilosis isolates. C. glabrata and C. krusei was intrinsically resistant to fluconazole.[5]

The changing antifungal spectrum of Candida blood stream infection has generated great concern about the emergence of drug-resistance strains of azole and its clinical outcome.

Antifungal susceptibility testing has been increasingly required in clinical practice. It is a well-known fact that the outcome of invasive fungal infections could be improved by early initiation of appropriate antifungal agent based on the susceptibility profile of infecting Candida species. Fully automated bioMerieux VITEK 2 system has expanded its role with a rapid yeast susceptibility test that determines Candida growth spectrophotometrically using VITEK 2 microbiology systems performing testing of susceptibility to flucytosine, amphotericin B, fluconazole and voriconazole.[6] Hence, this study was taken to determine the antifungal susceptibility profile of Candida isolates isolated from BSI by rapid automated bioMerieux VITEK 2 system to initiate early and correct antifungal treatment.

MATERIALS AND METHODS

This prospective study was conducted for a period of one year from May 2012 to May 2013 in the Department of Microbiology of a tertiary care hospital after obtaining the institutional ethics committee approval.

The study population was composed of all adult and paediatric-hospitalised patients of both genders who developed candidaemia. Candidaemia was defined as one or more blood cultures positive for Candida species in patients with relevant clinical signs and symptoms and risk factors.[7] A total of 50 Candida isolates were collected from BACTEC blood cultures of hospitalised patients. Candida strains from positive blood culture samples was identified by Gram stain and culture of the blood specimens on Sabouraud's dextrose agar, 5% sheep blood agar and chocolate agar and by colony morphology. Candida speciation and antifungal susceptibility testing was carried out on automated VITEK 2 system. The following strains were used as controls for the evaluation- C. albicans ATCC90028 and Candida tropicalis ATCC750.

Identification by VITEK 2 System- Pure subculture suspended in 3.0 mL of sterile saline of aqueous 0.45% NaCl (pH 4.5 to 7.0) was taken in a 12 x 75 mm clear plastic polystyrene test tube. The turbidity was adjusted to a McFarland 2.0 standard and measured on the DensiCHEK turbidity meter (bioMerieux, India), an instrument designed to measure the optical density of an organism suspension and measured using a turbidity meter called the DensiCHEK turbidity meter. The reading range of the DensiCHEK turbidity meter is 0.0-4.0 McFarland. The individual test cards were automatically filled with the prepared culture suspension, sealed and incubated by the VITEK 2 instrument. The cards were incubated at 35.5˚C for 18 hours and optical density readings were taken automatically every 15 minutes. The final profile results were compared with the database and the identification of the unknown organism was obtained.[8]

VITEK 2 Antifungal Susceptibility Testing Method- The turbidity of the overnight inoculum suspension from the culture was adjusted to 1.8-2.2 McFarland standard and this suspension was placed into the VITEK 2 cassette along with a sterile polystyrene test tube and an antifungal susceptibility test card for each organism. The VITEK 2 antifungal susceptibility cards containing serial two-fold dilutions of amphotericin B, fluconazole, flucytosine and voriconazole were used as provided by the manufacturer. The loaded cassettes were then placed into the VITEK 2 instrument. The inoculum suspensions were diluted appropriately by the instrument, then the cards were filled, incubated and read automatically by the instrument. The incubation time varies depending on the growth rate measured in drug-free control well. Quality control strains of C. albicans ATCC90028 and Candida tropicalis ATCC750 was used. According to the M27-A3 document, the results from the 48 hours reading were used. Complete data from the CLSI, EUCAST and VITEK 2 system for each fungal isolate were recorded.

Statistical Analysis- Statistical analysis was done in terms of frequency percentage, Fisher's exact test and the 'p' value was calculated to determine significance.

RESULTS

All the 50 isolates demonstrated sufficient growth at 16 hours of incubation and the species was determined by the VITEK 2 system at different incubation periods.

70% isolates were identified as non-albicans Candida and 30% of isolates was identified as C. albicans.

Among the 50 isolates, C. tropicalis was the predominant strain isolated in 22 (44%) isolates, followed by C. albicans in 15 (30%), C. glabrata in 7 (14%), C. krusei in 3 (6%), C. parapsilosis in 2 (4%) and C. kefyr in 1 (2%) isolate, respectively.

Species	Fluconazole		Voriconazole		Flucytosine		Amphotericin B	
	R	S	R	S	R	S	R	S
C. albicans (15)	0	15	0	15	0	15	0	15
C. tropicalis (22)	2	20	2	20	1	21	1	21
C. glabrata (7)	7	0	7	0	0	7	0	7
C. krusei (3)	3	0	1	2	0	3	0	3
C. parapsilosis (2)	0	2	0	2	0	2	0	2
C. kefyr (1)	0	1	0	1	0	1	0	1

Table 1. Antifungal Susceptibility Testing Pattern of the Candida Spp. Isolates

Fishers exact test, p=0.010, significant.

For fluconazole drug, VITEK 2 AST system showed that 76% isolates of Candida species were susceptible, while 24% were resistant. All the isolates of C. albicans (100%) and 76% of NAC were susceptible. C. glabrata and C. krusei were 100% resistant.

For voriconazole drug, VITEK 2 AST system showed that 80% isolates of Candida species were susceptible, while 20% were resistant. All the isolates of C. albicans (100%) and 80% of NAC were susceptible. C. glabrata and C. krusei were 100% and 33.3% resistant, respectively.

For flucytosine drug, VITEK 2 AST system showed that 98% isolates of Candida species were susceptible, while 2% were resistant. All the isolates of C. albicans (100%) and

98% of NAC were susceptible. C. tropicalis resistant percentage was 4.5%.

For amphotericin B drug, VITEK 2 AST system showed that 98% isolates of Candida species were susceptible, while 2% were resistant. All the isolates of C. albicans (100%) and 98% of NAC were susceptible.

The MIC of the two quality control strains was within the range of expected values and showed reproducibility by VITEK 2 AST.

All the C. albicans showed 100% susceptibility to fluconazole, voriconazole, flucytosine and amphotericin B. C. glabrata showed 100% resistance to azoles. C. krusei showed 100% resistance to fluconazole. 4.5% C. tropicalis showed resistance to amphotericin B.

DISCUSSION

C. tropicalis was the predominant strain isolated in 22 (44%) isolates followed by C. albicans in 15 (30%), C. glabrata in 7 (14%), C. krusei in 3 (6%), C. parapsilosis in 2 (4%) and C. kefyr in 1 (2%) isolate respectively indicating NAC as the common cause of fungal blood stream infection.

The most frequent species isolated from 275 patients in 5 years presented with candidaemia was C. tropicalis (35.3%), followed by C. albicans (21.5%), C. parapsilosis (20%), C. glabrata (17.5%), C. krusei (3.3%), C. haemulonii (1.5%) and C. guilliermondii (1%) according to study done by Xess et al[9] in North India.

In our study, 76% isolates of Candida species were susceptible and 24% were resistant to fluconazole. All the isolates of C. albicans (100%) and 76% of NAC were susceptible. C. glabrata and C. krusei were 100% resistant.

98.5% C. albicans isolates, 67% of C. glabrata and 26% of C. krusei isolates, 89.8% were susceptible to fluconazole in a study done by Jacques et al[10]

Kaur et al[11] showed that 92.4% isolates of the Candida species were susceptible. All the isolates of C. albicans were susceptible, while among NAC, 66.6% isolates were susceptible and remaining 33.4% were resistant in a study conducted on VITEK 2 system.

Voriconazole drug susceptibility showed that 80% isolates of Candida species were susceptible, while 20% were resistant. All the isolates of C. albicans (100%) and 80% of NAC were susceptible. C. glabrata and C. krusei were 100% and 33.3% resistant respectively in our study. Hence, we suggest voriconazole to treat yeast infections among fluconazole-resistant isolates except in case of C. glabrata and should be used cautiously in C. krusei infection.

94% of C. albicans isolates showed a good in vitro activity with voriconazole drug. C. glabrata was the least susceptible species and with the exception of C. glabrata, the MICs 90% for voriconazole were always ≤1 µg/mL suggesting that in most cases, this drug is effective to treat yeast infections among fluconazole or itraconazole-resistant isolates according to a study done by Danielle et al.[5]

Azoles are safe and effective agents for treatment of candidiasis and have gradually replaced amphotericin B. However, resistance to azoles is now becoming common. Several reports[12] suggest that susceptibility rates of Candida species to triazole antifungal amongst cancer patients have remained high with fluconazole resistance restricted to C. krusei and C. glabrata.

In our study, amphotericin B showed that 98% isolates of Candida species were susceptible, while 2% were resistant. All the isolates of C. albicans (100%) and 98% of NAC were susceptible. One isolate of C. tropicalis was resistant to amphotericin B in our study.

7% of C. albicans, 3.1% of C. krusei and 2.5% of C. glabrata were resistant to amphotericin B in a study done by Badiee et al.[13]

The different blood isolates were recovered from the neonates admitted in intensive care unit with high fever and respiratory distress. Nine of them received prolonged parenteral nutrition, while 2 were on fluconazole prophylaxis. Three neonates died in spite of an adequate antifungal therapy. The isolation of non-albicans Candida has been frequently encountered from candidaemic patients in the past few decades in a study conducted by Francisco et al.[14] In our NICU setting, most of the neonates were low birth weight and preterm and C. albicans was the predominant isolate.

In our study, 35 out of 50 isolates were non-albicans Candida. The emergence of C. glabrata as a common aetiological agent of candidaemia has important clinical implication due to its innate resistance to fluconazole. 12 out of 13 isolates from patients with candidaemia in a critical care unit were non-albicans mainly, C. krusei and C. tropicalis.[15]

IDSA guidelines have recently made significant changes for treatment of various infections caused by Candida. With the introduction of echinocandins, mainly the caspofungin, micafungin, anidulafungin and broad-spectrum azoles with lipid formulations of amphotericin B for the treatment of drug-resistant strains of Candida causing candidaemia, the morbidity and mortality is little on the downward trend.

The therapeutic options available for the management of invasive candidiasis and candidaemia have continued to increase with the addition of newer echinocandins and triazoles. For Candida glabrata and C. krusei infections, echinocandin is preferred.

Candida glabrata is intrinsically resistant to azoles and therefore azoles should not be preferred as the first line drug for treatment.[16]

In a study of clinical bloodstream, fungal isolates from Brazil by Melhem.[8] VITEK 2 system was used to identify fungal species and to determine antifungal susceptibility and was found to be in excellent correlation and agreement with the reference BMD methods by CLSI.

CONCLUSION

The successful treatment of Candida infections in blood depends on the rapid identification of the species and sensitivity patterns to antifungal agents. VITEK 2 system is a valuable tool for rapid identification and AST as it is rapid and less cumbersome, only disadvantage is the cost in some settings.

With the exception of C. glabrata, voriconazole is effective to treat most of yeast infections among fluconazole-resistant isolates.

Amphotericin B and voriconazole seem to be suitable drugs for empirical therapy in severe cases and fluconazole is not suitable, because most of the Candida species are resistance to them. Fluconazole treatment should be initiated based on antifungal susceptibility testing and speciation.

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