MINIMUM INHIBITORY CONCENTRATION OF VORICONAZOLE AGAINST FILAMENTOUS FUNGI FROM CLINICAL ISOLATES: A COMPARATIVE EVALUATION OF SENSITITRE® YEASTONE AND CLSI M38 METHOD.

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ABSTRACT

Voriconazole is a triazole antifungal to treat fungal infection. In this study, the susceptibility pattern of voriconazole against filamentous fungi was determined using Sensititre® YeastOne and Clinical & Laboratory Standards Institute (CLSI) M38 broth microdilution methods. The cultures of *Aspergillus niger, A. flavus, A. fumigatus, A. versicolor, A. sydowii, A. calidoustus, A. creber, A. ochraceopetaliformis, A. tamarii, Fusarium solani, F. longipes, F. falciferus, F. keratoplasticum, F. oxysporum, Talaromyces marneffei, Rhizopus oryzae, R. delemar, R. arrhizus, Mucor sp., Poitrasia circinans, Syncephalastrum racemosum* and *Sporothrix schenckii* were received from various government and private hospitals located all over Malaysia. The identities of the isolates were confirmed followed by their susceptibility testing via Sensititre® YeastOne and Clinical & Laboratory Standards Institute (CLSI) M38 broth microdilution methods. The significant differences between the two methods were calculated using Wilcoxon sign rank test. The comparative analysis between the two methods indicated a similarity in the MIC values of each isolate. The geometric mean of *Aspergillus spp., Fusarium spp.* and *T. marneffei* was within the range of 0.02 µg/ml- 2.00 µg/ml except *A. calidoustus, F. solani* and *F. keratoplasticum*. Similarly, the geometric mean of MIC for *S. schenkii* was around 3.00 µg/ml. Notably, the geometric mean of MIC for the members of *Zygomecete* class was ≥ 6.00 µg/ml. The general trend observed in MIC obtained by Sensititre® YeastOne was ±1 two-fold different compared with that obtained by the CLSI method. The overall agreement between the two methods to determine susceptibility testing of voriconazole was more than 70% except for *A. sydowii*. However, the differences between the two methods were significant when tested on *A. niger, A. flavus, A. fumigatus, A. versicolor, A. sydowii, F. solani* and *S. schenkii*. In conclusion, the Sensititre YeastOne method appears to be an alternative approach for voriconazole susceptibility testing for selected species of moulds isolated in Malaysia.

KEYWORDS: Susceptibility, voriconazole, Sensititre, CLSI, mould

INTRODUCTION

Voriconazole is a potent triazole drug with a broad spectrum of antifungal activity against many opportunistic fungal pathogens (Hitchcock et al. 1995; Radford, Johnson, and Warnock 1997; Sabo and Abdel-Rahman 2000; Saravolatz, Johnson, and Kauffman 2003; Greer 2003; Pearson et al. 2003). Its structure is similar to fluconazole with an addition of a methyl group to the propyl backbone and the substitution of a triazole moiety with a fluoropyrimidine group (Bruton, Chabner, and Knollomann 2006). In addition, its mechanism of action is to inhibit the cytochrome P450 (CYP 450)–dependent 14 -lanosterol demethylation. Subsequently, it impairs the biosynthesis of ergosterol essential for the synthesis of the cytoplasmic membrane and leads to the accumulation of 14-methyl sterols, which may disturb the tight association of acyl chains of phospholipids, weaken the functions of certain membrane-bound enzyme and ultimately stop the growth of the fungi (Bruton, Chabner, and Knollomann 2006).

Voriconazole can be administered via several routes such as intrastromal, intracameral and intravitreal injections besides systemic administration via oral and intravenous routes (Heralgi et al. 2016). Due to its enhanced clinical efficacy and minimal toxicity, it has been able to prevent or delay mortalities in infected animals and humans (Chandrasekar and Manavathu 2001; George, Miniter, and Andriole 1996; Murphy et al. 1997; Scott and Simpson 2007). The *in vitro* efficacy of an antifungal can be identified by determining its minimum inhibitory concentration (MIC) through susceptibility testing (Rex et al. 2001). The susceptibility test of filamentous fungi or moulds is outlined by the Clinical and Laboratory Standards Institute (CLSI) in M38 standard reference (Clinical and Laboratory Standards Institute 2017). However, this test is still limited due to the cost of antifungal reagents, lack of established breakpoints for moulds and laborious procedures (Nizam et al. 2016). Recently, a well-known commercial panel named Sensititre® YeastOne (Thermo Fisher Scientific, Cleveland, United States) is widely used in many routine microbiology laboratories due to its convenience and time-saving benefits (Siopi, Pournaras, and Meletiadis 2017; Li et al. 2020). It is a commercial colorimetric panel that contains dried serial dilutions of antifungal agents in a disposable tray used to determine MIC (Castro et al. 2004). Moreover, the MIC is based on the visible colour change caused by an oxidation-reduction indicator named Alamar Blue (Sánchez-Sousa et al. 1999).

In this study, MICs of voriconazole against Malaysian mould isolates were determined as voriconazole is one of the common antifungals widely prescribed by physicians in Malaysian hospitals. This data can assist clinicians in monitoring and selecting appropriate therapy for patients. The present study also assessed the agreement between Sensititre® YeastOne and Clinical and Laboratory Standards Institute (CLSI) broth microdilution method M38 via *in vitro* susceptibility testing of voriconazole against Malaysian mould isolates.

MATERIALS AND METHODS

Sample

A total of 100 clinical mould samples on potato dextrose agar (PDA) plates were received from various local and private hospitals in Malaysia in the year 2020. Majority of the cultures received were identified as *Aspergillus niger* (n=24), however, others includes *A. flavus* (n=13), *A. fumigatus* (n=12), *A. versicolor* (n=8), *A. sydowii* (n=4), *A. calidoustus* (n=3), *A. creber* (n=1), *A. ochraceopetaliformis* (n=1), *A. tamarii* (n=1), *Fusarium solani* (n=6), *F. longipes* (n=2), *F. falciferus* (n=1), *F. keratoplasticum* (n=3), *F. oxysporum* (n=1), *Talaromyces marneffei* (n=2), *Rhizopus oryzae* (n=2), *R. delemar* (n=1), *R. arrhizus* (n=1), *Mucor sp.* (n=2), *Poitrasia circinans* (n=1), *Syncephalastrum racemosum* (n=2) and *Sporothrix schenckii* (n= 9). Their identifications were confirmed by both macroscopic and microscopic methods. However, amplifying and sequencing of the internal transcribed spacer (ITS) region was performed for selected isolates that were not been able to detect by macroscopic and microscopic methods (Schoch et al. 2012).

Briefly, moulds were cultured on PDA plates and incubated at 30 °C except for *S. schenkii* and *T. marneffei* which were incubated at 25 °C (Clinical and Laboratory Standards Institute 2017). The growth of fungal colonies was inspected regularly. The lactophenol cotton blue wet mount was used to stain the mature colony with the scotch-tape technique before examining under microscope.

Culture medium

RPMI-1640 (Sigma-Aldrich, St. Louis, United States) with glutamine and phenol red, without sodium bicarbonate and buffered with 0.165 mol/L 3-morpholinopropanesulfonic acid (MOPS) (Sigma-Aldrich, St. Louis, United States) at pH 7.0, was used as the basal medium. RPMI-1640 was prepared as per CLSI M38 (Clinical and Laboratory

Standards Institute 2017).

CLSI method

Voriconazole (VOR) (Pfizer, North Carolina, USA) was dissolved with Dimethyl sulfoxide (DMSO) (Sigma-Aldrich, St. Louis, United States) and diluted in RPMI media as mentioned in CLSI M38 (Clinical and Laboratory Standards Institute 2017). Antifungal dilutions were ranged from 0.0625 to 32 µg/ml. For the reference broth microdilution testing, conidial suspensions were prepared as described in the CLSI M38 document (Clinical and Laboratory Standards Institute 2017). The plates were incubated at 30 °C except for *S. schenkii* and *T. marneffei* that were incubated at 25 °C. The incubation period and MIC was recorded visually according to the CLSI M38 document (Clinical and Laboratory Standards Institute 2017). MIC was defined as the lowest drug concentration that prevents any discernible growth.

Sensititre YeastOne

A colorimetric microdilution method was performed using commercially available Sensititre® YeastOne panels (Thermo Fisher Scientific, Cleveland, United States) according to the manufacturer's instructions. The colony-forming unit was confirmed each time by spread plate counts on PDA plates. The range of concentration for voriconazole in the panel was 0.008 to 8 µg/ml. Results were read by observing the lowest antifungal concentration with inhibition of growth, or no colour change (blue).

Quality controls

Each test was included with two reference strains; *A. flavus* ATCC 204304, *A. fumigatus* ATCC 204305, to ensure that the MIC obtained fall within the reference range.

Analysis of data

The range and geometric mean MIC were calculated using Microsoft Excel 2019 software for each species and method. In addition, MIC_{50}/MIC_{90} for species that have at least two isolates were calculated as well. MIC_{50} and MIC_{90} values were defined as the lowest concentration of the antibiotic at which 50% and 90% of the isolates were inhibited, respectively (Yamazhan et al. 2005). Moreover, the 2-fold differences and the level of agreement between the two methods were calculated as the proportion of the Sensititre® YeastOne colour endpoints determined for each strain that fell within ±1 twofold dilutions of the corresponding MICs of the CLSI method. Finally, the significant differences between two methods were calculated using the Wilcoxon sign rank test. These tests were performed only for species that have more than three isolates. *P*-values less than 0.05 were considered statistically significant.

RESULTS

In vitro susceptibility testing was carried out using voriconazole against *Aspergillus spp.* (n=67), *Fusarium spp.* (n=13), *T. marneffei* (n=2), R*hizopus spp.* (n=4), *Mucor sp.* (n=2), *P. circinans* (n=1), *S. racemosum* (n=2), and *S. schenckii* (n= 9). The MIC range, geometric mean, MIC_{50} and MIC_{90} results for both Sensititre® YeastOne and reference CLSI method are shown in Table 1. The *Aspergillus spp.* were the most commonly isolated molds among all the samples received. The geometric mean MICs of voriconazole against *Aspergillus spp.* were ≤2 µg/ml, with exception of *A. calidoustus*, which recorded 8 µg/ml with Sensititre® YeastOne; while 4 µg/ml with CLSI reference method. Interestingly, the agreement between the two methods was high (≥90%) when tested on *A. niger, A. flavus, A. fumigatus, A. calidoutus, A. creber, A. ochraceopetaliformis, A. ochraceopetaliformis* and *A. tamarii.*

However, the geometric mean MICs of voriconazole for *Fusarium spp.* were slightly higher than most of the *Aspergillus spp.* The minimum and maximum geometric mean MIC for *Fusarium* were 0.50 µg/ml and >8.00 µg/ml respectively. Notably, selected isolates of *F. solani* and *F. keratoplasticum* have higher MIC (≥8.00 µg/ml) compared with *F. longipes, F. falciferus* and *F. oxysporum* which have lower MIC (≤2.00 µg/ml). In general, Sensititre MIC_{50} and MIC_{90} values were a 2-fold dilution higher or lower than those from CLSI. Finally, the percentage of agreement between the two methods for most species was 100% and it was higher compared with *Aspergillus spp*.

On the other hand, the geometric mean MIC for *T. marneffei* was the lowest compared to other moulds. The agreement between the two methods for this species was unable to be calculated as the CLSI reading exceeded the tested range. All the isolates of *Rhizopus spp*. showed similar results when tested by Sensititre or CLSI method. The geometric mean MICs of voriconazole for *Rhizopus spp*. were higher than *Aspergillus spp.* and most of the *Fusarium spp.* However, the exact MICs for *Rhizopus spp*. could not be determined by Sensititre as they exceeded the tested range, resulting in failure to determine the MIC_{50} and MIC_{90}. This scenario is despite the observation of achieving 100% agreement between

the two methods.

Similar to *Rhizopus spp*., the geometric mean MICs of voriconazole against *Mucor sp*., *P. circinans, S. racemosum* and *S. schenkii* were higher than most of the *Aspergillus spp.* and *Fusarium spp*. The MIC values by the Sensititre method were a double dilution different than those of the CLSI method, except *S. schenkii* which has recorded a similar value. One of each *Mucor sp.* and *P. circinans* species were found unable to be inhibited even at the highest tested concentration of voriconazole by both methods. However, the percentage of agreement for both methods to determine MIC against *S. schenkii* (77.78%) was lower than *Mucor sp., P. circinans,* and *S. racemosum* (100%).

DISCUSSION

Voriconazole is a second-generation triazole antifungal agent with enhanced antifungal activity (Saravolatz, Johnson, and Kauffman 2003; Bow and Bacteriol 2009). It has good bioavailability where 96% of oral bioavailability; 56% of protein binding; extensive drug distribution in tissues (4.6 L/kg) and less than 2% of the unmodified drug is excreted in the urine (Heralgi et al. 2016; Patterson and Coates 1995).

Several clinical studies have evaluated the efficacy of voriconazole against invasive aspergillosis and found encouraging results (Denning, del Favero, and Gluckman 1995; Dupont et al. 1995). The initial results from animal trials suggested voriconazole was effective in the treatment of disseminated *Aspergillus* infection (George, Miniter, and Andriole 1996; Martin, Yates, and Hitchcock 1997). Moreover, it has also been reported to be effective in the treatment of invasive aspergillosis in children and adults (Scott and Simpson 2007). Interestingly, the superiority of voriconazole to amphotericin B for the treatment of invasive aspergillosis also has been reported (Herbrecht et al. 2002; Nivoix et al. 2008). Following that, voriconazole was approved by the Food and Drug Administration for the treatment of invasive aspergillosis (Greer 2003).

The breakpoints of voriconazole against moulds have not been determined by CLSI (Clinical and Laboratory Standards Institute 2017). However, European Committee on Antimicrobial Susceptibility Testing Subcommittee on Antifungal Susceptibility Testing (EUCAST-AFST) had determined the latest breakpoints for voriconazole against *A. fumigatus* where susceptibility ≤1 mg/L and resistance >1 mg/L (European Committee on Antimicrobial Susceptibility Testing 2020). The voriconazole MICs for *Aspergillus* showed no significant difference among species (Arikan et al. 1999). This is parallel with our finding except for A. calidoustus. In addition, the ratio of Sensititre MIC_{50} to MIC_{90} for *A. niger* and *A. flavus* in this study were two-fold higher than Linares et al. (2005); however, the CLSI ratio for *A. fumigatus* and *A. niger* were same. On the other hand, the ratio of CLSI MIC_{50} to MIC_{90} of A. niger was similar to the study conducted by Murphy et al. (1997); however, it was observed to be lower for *A. fumigatus* and *A. flavus.* Likewise, the CLSI mean and MIC_{90} for *A. fumigatus, A. niger* and *A. flavus* reported by Espinel-Ingroff were two-fold higher than our finding (Espinel-Ingroff 2001).

Fusarium spp. are resistant *in vitro* to many antifungal compounds (Alastruey-Izquierdo et al. 2008). Voriconazole is approved for the treatment of *Fusarium* infections in patients who are intolerant of or not responding to other drugs (Bow and Bacteriol 2009). However, the satisfactory response for voriconazole against fusariosis was just 45% (Perfect et al. 2003). The MIC of voriconazole against *Fusarium spp*. was higher than other moulds including *Aspergillus spp.* (European Committee on Antimicrobial Susceptibility Testing 2020; Lalitha, Shapiro, and Srinivasan 2007). Arikan et al. (1999) suggested that it might be due to the use of 100% growth reduction endpoint instead of 50% (Arikan et al. 1999). The MIC of *Fusarium spp.* was usually ranged from 1 to 4 µg/ml (Arikan et al. 1999; Lalitha, Shapiro, and Srinivasan 2007). However, among several tested *Fusarium spp*., *F. solani* was found to be the most resistant species to various drugs including amphotericin B, itraconazole, posaconazole and voriconazole (Cuenca-Estrella et al. 2006). The MIC_{50} and MIC_{90} of voriconazole against *F. solani* were recorded as >8.0 µg/ml. Similar findings were obtained by Alastruey-Izquierdo et al. where their MIC was ranged from 4 to 16 µg/ml (Alastruey-Izquierdo et al. 2008). Interestingly, their findings are similar to our observations except for one of the samples which showed a low MIC value recorded as 0.50 µg/ml.

Talaromyces marneffei was formerly known as *Penicillium marneffei* (Lau, Tsang, and Woo 2017). It is commonly associated with HIV-positive patients in Southeast Asia (Vanittanakom et al. 2006). The susceptibility patterns of this mould are not been extensively reported. Parallel to our findings, voriconazole had shown active activity against *T. marneffei* in the previous two reports. The mean value of MIC was observed to be low in previous literature,

Table 1. MIC of moulds to voriconazole and agreement between Sensititre YeastOne and CLSI broth microdilution method

Species	MIC by Sensititre® YeastOne (µg/ml)			MIC by CLSI (µg/ml)			Agreement (± one 2-fold dilution) (%)	p-value
	Range	Geometric Mean	MIC^{50}/ MIC^{90}	Range	Geometric Mean	MIC^{50}/ MIC^{90}		
A. niger (n=24)	0.03-2.00	0.46	0.50/ 1.80	<0.03-1.00	*	0.25/ 0.50	91.67	*
A. flavus (n=13)	0.25- 1.00	0.62	0.50/ 1.00	0.25- 1.00	0.53	0.50/ 1.00	92.31	0.887
A. fumigatus (n=12)	0.25- 1.00	0.40	0.50/ 0.50	0.13- 1.00	0.31	0.25/ 0.50	91.67	0.439
A. versicolor (n=8)	0.03- 2.00	0.50	0.75/ 2.00	0.25- 2.00	1.00	1.00/ 2.00	75.00	0.443
A. sydowii (n=4)	1.00- 2.00	1.19	1.00/ 1.70	0.25- 1.00	0.50	0.50/ 0.85	50.00	0.180
A. calidoustus (n=3)	8.00	8.00	8.00/ 8.00	4.00	4.00	4.00/ 4.00	100.00	0.250
A. creber (n=1)	0.50	0.50	-	1.00	1.00	-	100.00	-
A.ochraceo-petaliformis (n=1)	0.50	0.50	-	1.00	1.00	-	100.00	-
A. tamarii (n=1)	0.25	0.25	-	0.50	0.50	-	100.00	-
F. solani (n=6)	0.50- 8.00	2.83	4.00/ 6.00	0.50-16.00	3.56	4.00/ 12.00	100.00	0.180
F. longipes (n=2)	2.00	2.00	2.00/ 2.00	0.50- 1.00	0.71	0.75/ 0.95	100.00	-
F. falciferus (n=1)	2.00	2.00	-	1.00	1.00	-	100.00	-
F. keratoplasticum (n=3)	2.00- >8.00	*	>8.00/ >8.00	2.00- 8.00	5.04	8.00/ 8.00	*	*
F. oxysporum (n=1)	2.00	2.00	-	1.00	1.00	-	100.00	-
T. marneffei (n=2)	0.015-0.03	0.02	0.02/0.03	< 0.03	*	*	*	-
R. oryzae (n=2)	8.00- >8.00	*	8.00/ >8.00	8.00- 8.00	8.00	8.00/ 8.00	100.00	*
R. delemar (n=1)	>8.00	*	-	16.00	16.00	-	100.00	-
R. arrhizus (n=1)	>8.00	*	-	16.00	16.00	-	100.00	-
Mucor sp. (n=2)	8.00- >8.00	*	*	4.00- >16.00	*	*	100.00	-

P. circinans (n=1)	>8.00	*	-	>16.00	*	-	100.00	-
S. racemosum (n=2)	8.00	8.00	8.00/ 8.00	4.00-8.00	5.66	6.00/ 7.66	100.00	-
S. schenkii (n=9)	0.50- 8.00	3.28	4.00/ 4.00	1.00- 8.00	2.78	2.00/ 4.00	77.78	0.302

* Unable to be calculated as the reading was exceeded the tested range.
- Not performed as the number of isolates did not meet the minimum requirement.

Abbreviations used in Table 1. MIC: Minimum inhibitory concentration, CLSI: Clinical and Laboratory Standards Institute, *A. niger: Aspergillus niger; A. flavus: Aspergillus flavus; A. fumigatus: Aspergillus fumigatus; A. versicolor: Aspergillus versicolor; A. sydowii: Aspergillus sydowii; A. calidoustus: Aspergillus calidoustus; A. creber: Aspergillus creber; A. ochraceopetaliformis: Aspergillus ochraceopetaliformis; A. tamarii: Aspergillus tamarii; F. solani: Fusarium solani; F. longipes: Fusarium longipes; F. falciferus: Fusarium falciferus; F. keratoplasticum: Fusarium keratoplasticum; F. oxysporum: Fusarium oxysporum; T. marneffei: Talaromyces marneffei; R. oryzae: Rhizopus oryzae; R. delemar: Rhizopus delemar; R. arrhizus: Rhizopus arrhizus; Mucor* sp.*: Mucor* species*; P. circinans: Poitrasia circinans; S. racemosum: Syncephalastrum racemosum; S. schenkii: Sporothrix schenckii*

where 0.125 µg/ml and 0.04 µg/ml were obtained by Singh and Devi (2018) and Liu, Liang, and Chen (2013) respectively. The effectiveness of voriconazole against *T. marneffei* was further demonstrated in the clinical studies carried out by Ouyang et al. (2017) and Ge et al. (2019).

Besides that, Rhizopus can also cause severe and fatal infections in immunocompromised patients (Petrikkos and Drogari-Apiranthitou 2011; Suthananthan, Koek, and Sieunarine 2017). The treatment of zygomycosis is problematic and frequently associated with suboptimal therapeutic outcomes (Greenberg et al. 2004). Previous studies have reported that voriconazole possessed no meaningful activity against *Rhizopus* strains (Dannaoui et al. 2003; Singh, Rimek, and Kappe 2005; Arikan et al. 2008), as parallel to our findings. Instead, posaconazole and amphotericin B were found active against Rhizopus (Arikan et al. 2008; Sun et al. 2002). The MIC from their combination was lower than those from single drug (Arikan et al. 2008). Therefore, their combinations can lead to further testing in future studies.

Similar to *Rhizopus spp.*, voriconazole was not found to be active against *Mucor spp*. (Dannaoui et al. 2003; Sun et al. 2002). As mucormycosis is less common than aspergillosis and the course is progressively rapid; therefore, the effectiveness of antifungal treatment in a small study is difficult to evaluate. Since Mucorales are resistant *in vitro* to many antifungals (Almyroudis et al. 2007), their treatment with fluconazole, flucytosine, ketoconazole, echinocandins, itraconazole and voriconazole were reported to be ineffective in many cases (Parthiban et al. 1998; Ribes, Vanover-Sams, and Baker 2000; Imhof et al. 2004; Vigouroux et al. 2005; Kontoyiannis and Lewis 2011). To date, data on the antifungal susceptibility of *Mucorales spp*. are limited, and MIC testing remains investigational (Kontoyiannis and Lewis 2011). The mean value of voriconazole MIC was higher than 32 µg/ml (Dannaoui et al. 2003; Sun et al. 2002) and the MIC_{90} was higher than 64 µg/ml (Sun et al. 2002). These MICs values were much higher as compared to our findings. However, more samples are needed to determine the accuracy of the result.

Poitrasia circinans has fall under the order Mucorales (Kontoyiannis and Lewis 2005). Limited information regarding its susceptibility or treatment is available. However, in this study, voriconazole was found to be inactive via both Sensititre and CLSI methods against *P. circinans*. These results were related to *Mucor* sp.

S. racemosum is an opportunistic pathogen and rarely caused infection in humans (Ribes, Vanover-Sams, and Baker 2000). Thus, research related to its susceptibility testing is limited. Chowdhary et al. reported that MIC_{50} and MIC_{90} were 8 µg/ml and 16 µg/ml respectively by the CLSI method which was observed to be four-fold higher than the CLSI results obtained in this study (Chowdhary et al. 2014).

Sporotrichosis is a subacute or chronic infection that caused by dimorphic fungus *Sporothrix schenckii* (Rodrigues et al. 2020). The antifungal drugs commonly used are itraconazole for cutaneous or lymphocutaneous fixed forms (de Lima Barros et al. 2011), and amphotericin B for disseminated cases (Yamada et al. 2011; Silva-Vergara et al. 2012). However, these antifungal drugs are not always efficient and may lead to chronicity in immunocompromised patients (Marimon et al. 2008). Several studies have investigated for alternatives including voriconazole; however, the MIC_{50} and MIC_{90} obtained were varied among them. By using CLSI method, MIC_{50} was reported in the range of 8 to 32 µg/ml; while the MIC_{90} was reported in the range of 16 to 32 µg/ml (Córdoba et al. 2018; Marimon et al. 2008; Rodrigues et al. 2014). Interestingly, both of the CLSI MIC50 and MIC90 in this study were much lower than these reported findings.

Although broth microdilution methods have improved the level of interlaboratory agreement of antifungal MIC endpoints; however, these procedures are tedious, inconvenient, and labour-intensive for clinical laboratories (Castro et al. 2004; Espinel-Ingroff et al. 1996; Guinea et al. 2006). The need to prepare microdilution plates for the M38 method is indeed time-consuming and impractical for routine use in clinical microbiology laboratories (Wang et al. 2018). Sensititre® YeastOne is an adapted susceptibility system of the microbroth dilution CLSI method based on the M27-A3 standard for yeasts. Although it has been approved by the U.S. Food and Drug Administration (FDA) for *Candida* species (Pfaller 2012), its efficacy for the susceptibility determination of filamentous molds is an interesting aspect to further investigate. Hence, this study can act as a fundamental breakthrough for the comparative analysis of the Sensititre® YeastOne and conventional CLSI method against Malaysian mould isolates.

The level of agreement for voriconazole between Sensititre and CLSI methods was inconsistent in previous literature. For example, Wang et al. (2018) and Mello et al. (2017) had found a 100% agreement of Sensititre with the CLSI reference method for the

voriconazole when they tested on several *Aspergillus spp* (Wang et al. 2018; Mello et al. 2017). In contrast, Castro et al. reported that the overall agreement between Sensititre and CLSI methods for voriconazole was only 82.5% (Castro et al. 2004). Moreover, the phenomenon of the Sensititre® YeastOne test tended to increase or decrease the MIC by one dilution when compared with the reference (Siopi, Pournaras, and Meletiadis 2017; Castro et al. 2004; Sánchez-Sousa et al. 1999; Guinea et al. 2006). Data derived from the present study support the claim that the Sensititre® Yeast One method is equivalent to the CLSI reference method for the determination of MIC of A. flavus, *A. fumigatus, A. versicolor, A. sydowii, A. calidoustus, F. solani* and *S. schenkii* against voriconazole. This was further verified by Wilcoxon signed-rank test which was found to be insignificant ($p > 0.05$) between the two methods. Hence our results are consistent with previous studies (Castro et al. 2004; Guinea et al. 2006; Martin-Mazuelos et al. 2003).

To our knowledge, this is the first study to compare the susceptibility of voriconazole against Malaysian mould isolates using both CLSI and commercial Sensititre® YeastOne methods. However, there are several limitations in this study. The sample sizes of some species were small and thus MIC_{50} and MIC_{90} were unable to be determined. In addition, the MICs were still not able to be interpreted as susceptible or resistant there are no official clinically correlated breakpoints for moulds according to CLSI method. Nevertheless, these results could contribute to its limited antifungal database in Malaysia.

CONCLUSION

In conclusion, voriconazole possesses reliable antifungal activity against most of the moulds tested in this study except *A. calidoustus, F. keratoplasticum, R. oryzae, R. delemar, R. arrhizus, Mucor sp., P. circinans, S. racemosum* and *S. schenkii* as their geometric mean MICs were more than 1 µg/mL. In addition, Sensititre® Yeast One method can be used as an alternative approach to CLSI reference for the determination of MIC for voriconazole against *A. flavus, A. fumigatus, A. versicolor, A. sydowii, A. calidoustus, F. solani* and *S. schenkii*. However, this study can be further validated using a larger sample size.

ETHICAL DECLARATIONS

Ethical review was conducted and approved by the Medical Research and Ethics Committee, Ministry of Health of Malaysia, Malaysia (NMRR-20-207-53607).

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