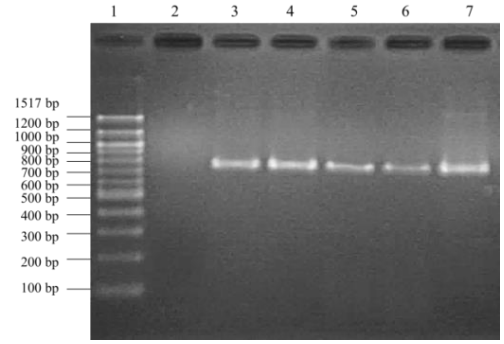

Figure 1 show the amplification products of *URA5* gene, *lane* 1 is the molecular marker (DNA ladder 100 bp), *lane* 2 is the negative control (DDW), *lane* 3 is the positive control (References strain WM 148) and *lans* 4, 5, 6 , 7 are clinical isolates of *C. neoformans*.

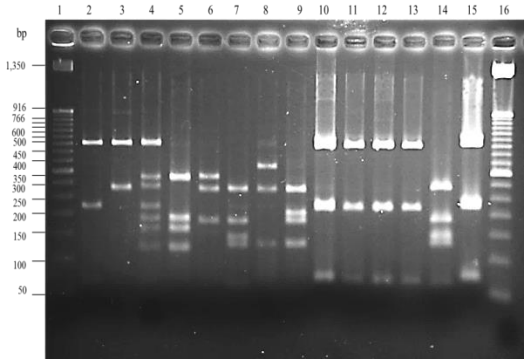

Figure 2 The PCR- RFLP profile of the *URA5* gene from *Cryptococcus* spp. Obtained double digested with *Sau*96I and *Hha*I. *Lanes* 1 and 16 are the molecular marker (DNA ladder 50 bp), *lanes* 2, 3, 4, 5 are genotype VNI, VNII, VNIII, VNIV, respectively. *Lanes* 6, 7, 8, 9 are the genotype VGI, VGII, VGIII and VGIV and *lanes* 10, 11, 12, 13, 14, 15 are *C. neoformans* clinical isolates from patients.

The *in vitro* susceptibility profile of *C. neoformans* 36 isolates to six antifungal agent drugs, MIC range, MIC_{50}, MIC_{90} and MIC geometric mean (µg/ml) were showed in table 2. We found that the susceptible to fluconazole, voriconazole, and posaconazole were 100% inhibition by concentration of 8 µg/ml, 0.12 µg/ml and 0.25 µg/ml, respectively and the results of itraconazole and 5- flucytosine were presented 97.22 (35/36) and 94.44% (34/36) respectively. In addition, there are twenty-nine from thirty-six isolates were susceptible to amphotericin B but only 7 resistant isolates.

Discussion and conclusion

The molecular epidemiology has been studied in worldwide. The main etiology agents cryptococcosis are two species *C. neoformans* and *C. gatti* that present numerous differences geographical distribution, epidemiology and molecular character (Kaocharoen et al., 2013). Genotyping of pathogen species complex have been recognized VNI, VNII, VNB, VNIII and VNIV among *C. neoformans* isolates and VGI, VGII, VGIII and VGIV among *C. gattii* isolates from previous studies (Bertout et al., 2013; Ferreira-Paim et al., 2011). At that time, the molecular types VNI and VGII were founded the most common genotype worldwide. In this study, molecular type VNI (98.57%) being the most common molecular type and the rare molecular type VGII (1.43%) which only one case was founded. Previously study described *C. gattii* isolates belong to the VGII genotype (Trilles et al., 2008). This genotype was reported as the cryptococcosis outbreak that occurred on Vancouver Island (BC, Canada)

Table 2 MIC value, geometric mean and susceptibility profile of *C. neoformans* isolates *N* =36

Antifungal agent	MIC range (µg/ml)	MIC_{50} (µg/ml)	MIC_{90} (µg/ml)	MIC geometric mean (µg/ml)	Susceptibility profile % (isolates)			
					S	S-DD	I	R
Amphotericin B	0.5-1	0.5	1	0.583	80.56 (29/36)	0	0	19.44 (7/36)
Fluconazole	2-8	4	8	4.16	100 (36/36)	0	0	0
Itraconazole	0.03-0.25	0.12	0.25	0.084	97.22 (35/36)	2.78 (1/36)	0	0
Voriconazole	0.015-0.12	0.12	0.12	0.049	100 (36/36)	0	0	0
5-Flucytosine	1-8	4	8	2.774	94.44 (34/36)	0	5.56 (2/36)	0
Posaconazole	0.03-0.25	0.12	0.25	0.090	100 (36/36)	0	0	0

MIC, Minimum Inhibitory Concentration MIC_{50}, MIC at which 50% of the isolates are inhibited MIC_{90}, MIC at which 90% of the isolates are inhibited *S*, susceptible *S-DD*, susceptible dose-dependent *I*, intermediate *R*, resistant

(Byrnes et al., 2009). In Brazil reported of this VGII genotype, it's potential to cause severe disease in immunocompetent host (Matos et al., 2012). The determination of susceptible profile revealed *C. neoformans* isolates to antifungal drugs, studies conducted in several part of the world have shown low MIC_{50} and MIC_{90} values for fluconazole against *C. neoformans* VNI (Tay et al., 2006). In this study no evidence of resistant to fluconazole (100% susceptible at MIC_{90} 8 µg/ml), posaconazole, voriconazole (100% susceptible at MIC_{90} 0.25, 0.12 µg/ml) but itraconazole (97.22% susceptible at MIC_{90} 0.25 µg/ml, 2.78% susceptible, 5-flucytosine (94.44% susceptible at MIC_{90} 8 µg/ml). The majority of studies addressing *in vitro* susceptibility testing for amphotericin B against *C. neoformans* and *C. gattii* had showed that most of these isolates susceptible at MIC values $\leq$ 1 mg/L. Lozano-Chui and coworker (1998) reported MIC values of 3-4 mg/L, which were associated with treatment failure.

Identification of the species by PCR-RFLP and antifungal susceptible analysis are important to determining the prevalent of *C. neoformans* including therapy in patients. Moreover, we have knowledge the importance of performing molecular typing to give finding of difference in the *in vitro* susceptibility profile in Khon Kaen, Thailand.

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