O085
Oral Session
Emerging resistance in fungi

AZOLE RESISTANCE MECHANISMS FOUND AMONG FLUCONAZOLE-RESISTANT CANDIDA ALBICANS CLINICAL ISOLATES: REPORT FROM THE SENTRY ANTIFUNGAL RESISTANCE PROGRAMME

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Objectives: To investigate azole resistance mechanisms in five fluconazole-resistant *C. albicans* clinical isolates collected during 2011 and 2012 from hospitals participating in a large surveillance program. Studies to determine fluconazole resistance mechanisms are usually performed using laboratory-generated strains; and various mechanisms including target alteration overexpression of multidrug efflux pumps or genetic rearrangements have been described.

Methods: C. albicans isolates were susceptibility tested by CLSI broth microdilution methods. Mutations in Upc2, TAC1, Erg11, MDR1 and Mrr1 were screened by PCR/sequencing. Expressions of Erg11, CDR1, CDR2 and MDR1 were determined using high quality mRNA in triplicate reactions by quantitative RT-PCR normalized with an internal control gene. Homozygosis of Mrr1, MDR1 and MDR1 promoter region was investigated with a combination of restriction digestion followed by probe hybridization and shotgun cloning/sequencing of multiple recombinants. C. albicans ATCC 90028 was used as control.

Results: Among 1,582 C. albicans isolates, only 5 (0.3%) displayed fluconazole MIC values ranging from 8 to >128 mg/L. Isolates were collected in the USA (2), India, Spain and Argentina. Three fluconazole-resistant isolates were also resistant to itraconazole, voriconazole and posaconazole (MIC, >8 mg/L for all) and those displayed highest fluconazole MIC values (>=128 mg/L; 2 USA and 1 India strains). Upc2 was found to be homozygous for allele-1 in four isolates and for allele-2 in one strain (cross-resistant to all azoles), whereas the ATCC control was heterozygous. Mutations on TAC1 described to cause hyperactivity of other resistance genes were not observed. Erg11 mutations were detected in 4/5 isolates with no apparent correlation with azole cross-resistance. Only one strain had mutations on MDR1 and no mutations previously associated with azole resistance were observed in Mrr1. CDR1 overexpression was observed in three isolates (4.8, 6.2 and 18.5X greater than control) that also showed overexpression of CDR2 (17.4, 31.4 and 46.2X greater than the control strain). One strain displaying elevated MIC values to all azoles had CDR1 and CDR2, and modestly elevated expression of MDR1 (6.5X greater than control). Erg11 expression was basal in all isolates. Hybridization using Mrr1 probe revealed homozygosis in three isolates while cloning/sequencing showed polymorphisms in two isolates, demonstrating poor correlation of these assays. MDR1 homozygosis was observed in three isolates and promotor homozygosis, as well as -306 A/A genotype associated with resistant laboratory mutants was present in only one isolate.

Conclusions: Overexpression of efflux pumps CDR1, CDR2 and MDR1 most likely contributed to azole cross-resistance in at least one isolate in this collection. Two fluconazole-resistant isolates displaying modestly elevated MIC values (0.25 – 1 mg/L) for other azoles had elevated expression of CDR1 and CDR2. However, the two isolates from the USA had none of the resistance mechanisms investigated.