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Overexpression as a major genetic mechanism of fluconazole resistance in *Candida albicans* isolated from HIV patients in Indonesia

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Overexpression and mutations as a genetic mechanism of *Candida albicans* resistance to fluconazole have been reported. Mechanisms responsible for *Candida albicans* isolated from HIV patients in Indonesia are less understood. Overused of fluconazole for treatment of oropharyngeal candidiasis, which was the third most common opportunistic infection in HIV/AIDS patients in Indonesia could lead to the emergence of resistance. Result analysis of *ERG11, CDR1, CDR2* and *MDR1* by real-time RT-PCR and *ERG11* gene mutation using sequencing methods showed that the highest gene overexpression of *CDR2* was detected in all isolates of *C. albicans* resistant to multiple azoles, overexpression of *ERG11* gene that play a role in *C. albicans* isolates resistant to a single fluconazole. Although amino acid substitutions were observed at six positions, i.e. D116E, D153E, I261V, E266D, V437I and V488I, it seems not directly related to the fluconazole resistance. This shows that the genetic polymorphism lanosterol 14-α demethylase is highly permissive to structural changes. Amino acid substitution at I261V due to *ERG11* genes mutation identified in this study is probably associated with fluconazole resistance, since it is located at the β helical chain, the binding site of fluconazole and the substitution from the large size isoleucine amino acid to small size valine hinders the entry of fluconazole. However, the combination of overexpression of *CDR2* and *ERG11* and mutation in the *ERG11* gene were responsible as a genetic mechanism of fluconazole resistance in *C. albicans* isolated from HIV patients in Indonesia.

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Isolation and characterization of toxins produced by *Cronobacter* species and the subsequent virulence characterization of the toxin producing strains

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Cronobacter spp. is a group of Gram negative pathogens that have been implicated as the causative agents of necrotizing enterocolitis and meningitis in infants. Progress in understanding the virulence mechanism of *Cronobacter* has been poor to date and not much is known about the factors involved in its pathogenicity. The phylogenetic analysis based on 16S rRNA gene sequences of the 47 *Cronobacter* isolates used in this study identified four main clusters with *C. sakazakii* was the most represented species (78.72%) followed by *C. muytjensii* (14.89%), *C. turicensis* (4.26%) and *C. dublinensis* (2.13%). The antibiotic resistance profile of the isolates revealed a low resistance profiles with only 10.64%, 8.51% and 4.26% were resistant to ampicillin, cefoxitin and amoxicillin-clavulanic acid, respectively. Most of the isolates (76.6%) were susceptible to all the antibiotics used. PCR screening of putative virulence genes showed that *C. sakazakii* isolates harbor virulence genes more frequently than other *Cronobacter* species with siderophore interacting protein gene (*sip*) and iron acquisition gene clusters (*eitCBAD* and *iucABCD/iutA*) were the most detected genes. To address potential virulence factors and toxins produced by *Cronobacter*, the filtrates of the isolates were tested on cell culture. The results of Vero cells assay varied between the 47 isolates, whereas most isolates exhibited a weak cytotoxicity. Nine isolates were selected subsequently to be evaluated for enterotoxin production in suckling mice. The filtrates tested were consistently negative, even when two types of media (BHI and TSB) were used to culture the isolates. Concentrating the culture filtrates at least 20-fold using freeze drying showed a positive enterotoxin production for *C. sakazakii* 12C and *C. turicensis* Jor170. The toxin was purified from these strains by stepwise ammonium sulfate precipitation followed by Sepharose 6-B gel filtration chromatography. The molecular mass of the enterotoxin purified was determined to be around 40-50 kDa. Suckling mice were also challenged both intraperitoneally and orally to determine the minimum lethal dose for the nine isolates that were selected for enterotoxin assay. All the isolates caused death at 107 CFU per mouse when injected intraperitoneally, while seven isolates were lethal at the same dose by the peroral route. However, an obvious difference in infectivity between the strains was observed. Results obtained in this study might provide more answers about the virulence characteristics of *Cronobacter* species.

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