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عنوان:
(Sub-Fractional Inhibitory بررسی اثر غلظت زیر حد بازدارنده کسری رشد سفتازیمید در تركیب با ونوتوباپیلین یا کلیندامایسین بر برخی از Concentration) زندهای مؤثر در ویرولانسی، اسپورزابی و زایش اسپور کلستریدیوم دیفسیل
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The Effects of Sub-Fractional Inhibitory Concentration (Sub-FIC) of Ceftazidime in Combination with Vancomycin or Clindamycin on some of Virulence Genes, Sporulation and Spore Germination of Clostridium difficile

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Abstract

Introduction: Clostridium difficile (C. difficile) is an obligate, anaerobic spore-forming bacterium that infection is caused in the presence of antibiotic in gut. The bacterium consists virulence factors which may influence by the sublethal concentration of antibiotics. The effects of antibiotics in the sub-MIC have reported on the toxin production and virulence genes expression. It has been reported that C. difficile associated diarrhea have increased in the patients who received combination therapy.

In this research, besides the prevalence of C. difficile in the diarrhea samples which collected from intensive care unit (ICU) of Shahid Bahoonar hospital, Kerman, the effect of ceftazidime (CAZ) alone and in combination with vancomycin (VAN) and clindamycin (CLI) were evaluated in the sub-MIC on the spore production, spore germination, toxin A, B and slpA gene expression and toxin production of C. difficile.

Methods: About 233 diarrhea samples were gathered from diarrhea ICU hospitalized patients. The samples were cultivated on Clostridium difficile medium. Bacterial isolates were confirmed as C. difficile based on the odor, Sub-terminal spore, and 16S rDNA gene amplification. Four clinical isolates and one standard strain ATCC 9689 were selected for further investigations. MIC(VAN, CLI, and CAZ) and FIC (VAN plus CAZ and CLI plus CAZ) of four clinical isolates and ATCC 9689 strain were determined by microdilution and checkerboard microdilution method, respectively. The appropriate concentration of antibiotics alone (½ x MIC) and in combination (½ x FIC) were added to the tubes containing pre-reduced medium. Then ~10^6 CFU/mL fresh bacteria were inoculated into the tubes and incubated in anaerobic jar at 37 °C. The number of spores, toxin production and genes expression (tcdA, tcdB, and slpA) were carried out by colony count, ELISA and Real-time PCR methods in triplicate, respectively.

Results: From the 233 diarrreal samples were gathered from ICU, C. difficile isolated from 21% of them. Based on toxin assay by ELISA method, about 22.5% of C. difficile isolates were toxigenic. Four clinical isolates and ATCC 9689 strain were selected for further investigations. VAN inhibited spore production in the all isolates and ATCC 9689 strain. The other antibiotics alone and in combination suppressed spore production in majority of isolates (except A+/B+/CDT+). The antimicrobial agents down-regulated cdtA, cdtB and slpA genes expression in sub-MIC of antibiotics alone and in
combination within 24 hours periods. Down-regulation of genes expression continued in the sub-MIC of VAN, and CLI in the periods of 48 hours. At this time, CAZ and its combination with VAN and CLI increased cdtA, cdtB and slpA genes expression.

**Conclusion:** The frequency of *C. difficile* associated diarrhea in ICU hospitalized patients in this study was about 4.7% (11 cases) which is similar to Asia region and more than industrial countries reports. Cephalosporins such as CAZ are prescribed frequently for hospitalized patients. They are important agents in the induction of *C. difficile* infection (CDI). The results showed CAZ (alone and in combination) in comparison to VAN, and CLI increased toxins and slpA genes expression and confirms that cephalosporins with overexpression of virulence factors enhance the severity and spread of CDI. Meanwhile, CAZ interferes with the function of VAN and CLI. In the previous studies it has been shown that hospitals have reduced the use of cephalosporins, markedly decreased the incidence of CDI. Although, sporulation, germination, toxin production and virulence factors genes expression are related to the type of antibiotic and isolates.

**Keywords:** *Clostridium difficile*, Gene expression, Antibiotic, Germination and Sporulation.