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Inhibition of sessile and biofilm growth in various *Aspergillus* species by allicin associated with disruption to structural changes in cell wall

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Background: The alarming mortality rate of invasive aspergillosis, an emerging infection in immunocompromised hosts even with treatment with the best available antifungal drugs, warrants an alternative therapeutic regimen. Allicin (diallylthiosulphinate), which is extracted from *Allium sativum* (garlic), was shown to exhibit potent antimicrobial activities against various bacterial species and fungi. This study aimed to determine the antifungal effect of allicin against different species of filamentous fungi, *Aspergillus,* in the sessile and biofilm growth forms, and the ultrastructural changes on the cell wall due to *in vitro* allicin treatment.

Methods and materials: The effects of allicin against *Aspergillus* clinical isolates obtained from human patients were compared through the parameters of minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) according to the CLSI protocol. Commonly utilised antifungals, Amphotericin B (AmB) and fluconazole served as controls. Similarly, the effects of allicin towards fungal sessile growth and biofilm production were determined by XTT and crystal violet assay. Scanning electron microscopy (SEM) was performed to determine the effects of allicin on the cell wall architecture.

Results: The MIC obtained from clinically isolated *Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Aspergillus terreus* and *Aspergillus versicolor* treated with AmB indicated that the first three *Aspergillus* spp. were susceptible to treatment as the MIC was <2 μg/mL. Meanwhile, the MIC of *A. terreus* and *A. versicolor* were recorded to be 2 μg/mL. All five *Aspergillus* spp. tested against fluconazole exhibited growth at 256 μg/mL, indicating their susceptibility to fluconazole. On the other hand, allicin was shown to have potent antifungal activity against the five *Aspergillus* species at somewhat similar MICs and MFCs. XTT assay had also shown reduction in biomass while crystal violet assay demonstrated the decrease in biofilm biomass. The SEM results revealed remarkable disruptive effects of allicin upon the *Aspergillus* species cell wall.

Conclusion: The results here suggest that allicin could serve as a potential alternative treatment that is effective against common and not so common species of *Aspergillus* that cause human infections. Future studies could include combining allicin with nanoparticles for more targeted drug delivery.

https://doi.org/10.1016/j.ijid.2020.09.215

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Characterization of carbapenem-resistant *Acinetobacter baumannii* isolated from the intensive care unit in Malaysia

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Background: The emergence of carbapenem-resistant Acinetobacter baumannii (CRAB) has now become a global sentinel event. CRAB infections often instigate severe clinical complications and are potentially fatal, especially for debilitated patients. Due to the production of carbapenemase by this pathogen, carbapenem antibiotics have now fallen by the wayside, leaving colistin and tigecycline as the drugs of last resort for CRAB infections. The appearance of carbapenemase-producing strains, in addition to the biofilm-forming phenotype, constitute a major problem to the clinical environment. This study is aimed to determine the antibiotic resistance patterns, presence of OXA carbapanemase genes and the biofilm-forming capacity of CRAB isolated from various clinical specimens.

Methods and materials: A total of 100 CRAB strains isolated from year 2015 to 2016 were collected and their respective antimicrobial susceptibility patterns were determined using VITEK II system (bioMérieux), except for colistin which was determined using E-test. Isolates were screened for the presence of OXA carbapenemase and disinfectant-resistant genes through conventional polymerase chain reaction (PCR). Biofilm forming ability was investigated using quantitative adherence assay. In addition, the genetic relatedness of each strain was studied using Pulse-field Gel Electrophoresis (PFGE).

Results: Antimicrobial susceptibility testing showed that all isolates remained susceptible to colistin, even though 62% of them confer resistance to all other classes of antibiotics tested. Moreover, some isolates (38%) were still susceptible to antibiotics from class aminoglycoside and trimethoprim-sulfamethoxazole. All isolates showed the co-harbouring of $bla_{\text{OXA-23}}$-like and $bla_{\text{OXA-51}}$-like carbapenemase genes. Disinfectant resistance genes *qac*E and *qac*ΔE were not detected among them. In terms of biofilm formation, 72% ($n = 100$) of the isolates were biofilm formers. Among these, 64% demonstrated a weak biofilm forming phenotype, 6% are moderate biofilm formers and only 2 isolates showed strong biofilm formation. Genomic-subtyping characterized the strains into 13 pulsotypes.

Conclusion: The present study revealed that $bla_{\text{OXA-23}}$ is the predominant carbapenemase gene circulating in the hospital. High incidence of genetically identical CRAB infection and colonization suggests that CRAB has become endemic in the clinical setting. This highlights the importance of better controlling and monitoring of CRAB in the hospital to avoid their dissemination and occurrence of nosocomial outbreaks.

https://doi.org/10.1016/j.ijid.2020.09.216