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In Vitro Susceptibilities of Ocular *Bacillus cereus* Isolates to Clindamycin, Gentamicin, and Vancomycin Alone or in Combination

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A broth dilution assay was used to determine the in vitro susceptibilities of 10 ocular isolates of *Bacillus cereus* to clindamycin, gentamicin, and vancomycin both alone and in combination. The checkerboard technique was used to determine fractional inhibitory and bactericidal concentration indices for combinations of clindamycin-gentamicin and vancomycin-gentamicin.

Bacillus cereus has emerged as one of the most virulent bacteria to affect the eye, causing a destructive endophthalmitis following trauma or intravenous drug use (1, 2, 5, 8–10, 14–17, 21). Nevertheless, there is limited information available with respect to its antimicrobial agent susceptibility. Published data suggest that *B. cereus* isolates are susceptible in vitro to gentamicin, vancomycin, clindamycin, chloramphenicol, and erythromycin (1, 4, 12, 14, 17, 20). The purpose of the present study was to investigate the in vitro antimicrobial agent susceptibility of 10 ocular isolates of *B. cereus*. A checkerboard technique was used to assess the effects of clindamycin, gentamicin, and vancomycin, used both alone and in combination.

The microorganisms studied were 10 *B. cereus* isolates recovered from ocular tissue specimens submitted to the Sid W. Richardson Ocular Microbiology Laboratory. Five isolates were recovered from vitreous, two isolates were recovered from corneal tissue, and three isolates were recovered from lids and orbital tissues. The isolates were identified by using the Minitek system.

The antibiotics used were clindamycin hydrochloride, gentamicin sulfate, and vancomycin hydrochloride. These antibiotics were selected because of their activities against *B. cereus* as suggested by previous reports and because they are commonly used against serious ocular infections (3, 14, 20). The antibiotic concentrations ranged from 0.3 to 32 μg/ml in serial twofold dilutions. Each antibiotic was tested as a single agent in a broth dilution assay. The standard inoculum contained approximately 7.5×10^5 organisms in 1 ml of cation-supplemented Mueller-Hinton broth (BBL Bioquest, Cockeysville, Md.). Culture wells were incubated at 35°C for 24 h. Clindamycin-gentamicin and vancomycin-gentamicin were tested as combined agents by using a checkerboard technique. Controls were included in each determination. The MIC was the lowest concentration of antibiotic that completely prevented visible bacterial growth.

The MBC was determined by transferring 0.01-ml broth samples to Mueller-Hinton agar plates and incubating the plates at 35°C for an additional 24 h. The lowest concentration of antibiotic that prevented macroscopic microbial growth was regarded as the MBC.

The fractional inhibitory and bactericidal concentration (FIC and FBC, respectively) indices for clindamycin-gentamicin and vancomycin-gentamicin were derived by using a checkerboard technique (6). Synergy and antagonism were defined by using the established criteria of indices of <0.5 and >4.0, respectively.

MICs for 50% of strains (MIC_{50}s) and MIC_{90}s for clindamycin, gentamicin, and vancomycin were 0.5 and 1.0, 2.0 and 4.0, and 2.0 and 2.0 μg/ml, respectively. MBC_{50}s and MBC_{90}s for clindamycin, gentamicin, and vancomycin were 1.0 and 4.0, 2.0 and 4.0, and 2.0 and 8.0 μg/ml, respectively. The FIC indices for the combination of vancomycin-gentamicin ranged from 0.51 to 1.50. The FBC indices ranged from 0.16 to 1.50. Vancomycin-gentamicin demonstrated inhibitional and bactericidal synergy in 40% (4 of 10) of isolates. Antagonism was not noted with any isolate. The FIC indices for the combination of clindamycin-gentamicin ranged from 0.25 to 1.25. The FBC indices ranged from 0.13 to 1.00. Clindamycin-gentamicin demonstrated inhibitional synergy in 40% (4 of 10) and bactericidal synergy in 60% (6 of 10) of isolates. Antagonism was not noted with any isolate.

The effects of *B. cereus* endophthalmitis are devastating. In the more than 20 cases reported in the literature, useful vision has been preserved in only 1 case and enucleation of evisceration has been necessary in 76% (19 of 25) (1, 2, 5, 8, 9, 11, 13, 16–19, 21). The importance of early clinical suspicion, correct laboratory evaluation, and optimal therapy cannot be overstated. Despite the importance of selecting the optimal antimicrobial agent to eradicate this organism, little has been published regarding its in vitro susceptibility.

The MICs and MBCs reported here suggest that clindamycin, gentamicin, and vancomycin are all relatively effective against *B. cereus* as single agents. This effect exists for both inhibitory and bactericidal activity and for essentially all isolates tested. Clindamycin possesses a fourfold MIC_{50} advantage over either vancomycin or gentamicin. This advantage becomes less apparent, however, when the MIC_{90} or bactericidal activity is considered.

The data on combined antimicrobial agent effect directly compare the in vitro activity of clindamycin-gentamicin with that of vancomycin-gentamicin against *B. cereus*. Although synergy against some *B. cereus* isolates was demonstrated by both combinations, clindamycin-gentamicin demonstrated a slightly higher rate of bactericidal synergy than vancomycin-gentamicin (60 versus 40%). Neither combination demonstrated antagonism against any isolate tested.

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Earlier investigations have reported in vitro antibiotic susceptibilities for clinical isolates of *Bacillus* species to single agent therapies. Coonrad et al. (3) reported data for a large number of *Bacillus* spp.; however, their values were determined by a disk diffusion method. O'Day et al. (14) reported on six clinical *B. cereus* isolates, but they reported only mean susceptibility data and failed to include a description of their laboratory methods. Weber et al. (20) reported on the in vitro susceptibility of 54 clinical isolates of *B. cereus* to selected antimicrobial agents. Susceptibility testing was performed by both broth microdilution assay and disk diffusion testing, with values limited to single-agent inhibitory activity.

O'Day et al. (14) also suggested that the combination of clindamycin and gentamicin was synergistic in the treatment of *B. cereus*. This assumption was based on the clinical microbiologic success of combined therapy in an experimental rabbit model of endophthalmitis. This was not confirmed by fractional concentration indices or isobolograms.

According to the in vitro data for ocular isolates contained in this report, clindamycin, vancomycin, and gentamicin are all relatively effective single agents against *B. cereus*. Additionally, the combinations of clindamycin-gentamicin and vancomycin-gentamicin are synergistic or near synergistic for most *B. cereus* isolates. Clindamycin-gentamicin demonstrated bactericidal synergy for slightly higher number of *B. cereus* isolates than vancomycin-gentamicin did. This bactericidal effect may be important clinically in disease states such as endophthalmitis, in which the host immune response to infection results in end organ damage, and in immunocompromised hosts, who have been shown to be at risk of developing *B. cereus* infections (4, 7, 10, 12).

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