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Letter to the Editor

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Effect of Mg^{+2} Concentration in Mueller-Hinton Agar on the Susceptibility of Pseudomonas Aeruginosa to Levofloxacin

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The fluoroquinolone antibacterials are considered valuable broad-spectrum agents. The class as a whole is characterized by good antibacterial activity after oral administration and by better performance against gram-negative organisms than gram-positive pathogens. Quinolenes exert their antibacterial action via inhibition of type II topoisomerase DNA gyrase, an essential bacterial enzyme which alters the topology of double stranded DNA within the cell. Levofloxacin is more potent against gram-negative bacteria, and it exhibits better antipseudomonal activity and greater oral bioavailability (1-3).

Auckenthaler and colleagues reported that Mg concentrations in media influence the suscetibility of some gram-negative and gram-positive bacteria to nalidixic acid, ciprofloxacin, enoxacin, norfloxacin, ofloxacin and pefloxacin. We attempted to determine whether a higher magnesium concentration in Mueller-Hinton agar (MHA) influences the zone diameters of levofloxacin againts *P. aeruginosa* strains in the disk diffusion method (4).

The magnesium concentration in BBL MHA (H2DWFX) used throughout the study was 2.4 mg/L, having been quantified by atomic absorption spectroscopy by Cooper and colleagues (5). The magnesium ion concentration of the medium was increased to 15 mg/L by the addition of $MgSO_4$, which gave a similar concentration to the 12.5 mg/L which was determined for BBL Mueller - Hinton broth (Lot no: 1000E0DHWY). Fifteen *P. aeruginosa* isolates were studied. The control strain used throughout the study was *P. aeruginosa* ATCCC 27853. All *P. aeruginosa* isolates were adjusted to an optical density of 0.5 McFarland standard (10^8 cfu/ml) with sterile saline and then further diluted to achieve a final bacterial concentration of 10^7 cfu/ml. The antibiotic susceptibilities of *P. aeruginosa* isolates to levofloxacin (disk content 5 µg) were determined in both media. The depth of MHA was c. 4mm. The procedure for the disk diffusion test was that recommended by the NCCLS (6).

The result of the susceptibility testing of fifteen *P. aeruginosa* isolates to levofloxacin on BBL MHA and magnesium-supplemented MHA are shown in the Table. Student's t-test was used in the evaluation of the difference between the susceptibilities of clinical isolates of *P. aeruginosa* to levofloxacin on both media. All

Table: Susceptibility of 15 P. aeruginosa isolates to levofloxacin on media containing two different amounts of magnesium.

	Mean (SEM) zone diameters (mm)	
Antibiotics	MHA (Mg 2.4 mg/L)	MHA with Mg supplement (Mg 15mg/L)
Levofloxacin	22.8 (0.6)	20.4 (0.5)

P<0.005

experiments were carried out twice. When magnesium-supplemented MHA was used, the zone diameters of levofloxacin with all isolates including the control decreased ($P<0.005$). Similar experiments were carried out for ceftazidime and no statistically significant differences were observed for these on either medium ($P>0.05$)(data not shown).

It is clear that the magnesium concentration in MHA affects the activity of levofloxacin against *P. aeruginosa*. The mechanism of impaired activity in the presence of magnesium is unknown. Magnesium might interfere at at least two levels, either on the outer membrane or at the level of DNA-gyrase DNA interaction (5,7). These observations may result in false reports of resistance to levofloxacin and restrict the use of these antibiotics in the treatment of *P. aeruginosa* infection. In addition, while testing the susceptibility of quinolones, the concentration of magnesium in the medium should be standardized (8,9).

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