CORRESPONDENCE

Effect of zinc concentration in Mueller-Hinton agar on susceptibility of *Pseudomonas aeruginosa* to meropenem

Meropenem, like imipenem, is a highly potent carbapenem antibiotic active against a broad range of bacteria including *Pseudomonas aeruginosa* [1]. A study by Cooper and colleagues reported that zinc concentration in commercial media influenced the susceptibility of *P. aeruginosa* and other gram-negative bacilli to imipenem [2]. Similar results were reported by Daly *et al.* in 1997 [3]. We attempted to determine whether higher zinc concentrations in Mueller-Hinton agar (MHA) influence the zone diameters of meropenem and imipenem in the disk diffusion method.

The zinc concentration in the Oxoid MHA (CM337B) used throughout the study was 0.45 mg/L having been quantified by Unipath (Basingstoke, Hants) by atomic absorption spectroscopy. The zinc ion concentration of the medium was increased to 3 mg/L by the addition of zinc acetate which gave a similar concentration to the 2.6 mg/L which was determined for BBL MHA (H2DWFX) [2].

Sixteen *P. aeruginosa* isolates were studied; the control strain used throughout the study was *P. aeruginosa* ATCC27853. All *P. aeruginosa* isolates were adjusted to an optical density of a 9.5 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 meropenem (disk content 10 µg) and imipenem (disk content 10 µg) were determined with both media. The depth of MHA was *c.* 4 mm. The procedure for the disk diffusion test was that recommended by the NCCLS [4].

The results of the susceptibility testing of 16 *P. aeruginosa* isolates to meropenem and imipenem on Oxoid MHA and zinc-supplemented Oxoid MHA are shown in Table 1. Student's *t*-test was used in the evaluation of the difference between the susceptibilities of clinical isolates of *P. aeruginosa* to meropenem and imipenem on both media; $p < 0.05$ was considered to be significant.

When zinc-supplemented MHA was used, the zone diameters of meropenem and imipenem with the 16 *P. aeruginosa* isolates and the control organism decreased ($p < 0.05$). Similar experiments were carried out for ceftazidime and piperacillin. No statistically significant differences were observed for these on

Table 1. Susceptibility of 16 *P. aeruginosa* isolates to meropenem, imipenem, ceftazidime and piperacillin on (media containing two different amounts of zinc)

Antibiotic	Mean (SEM) zone diameter with	
	MHA (zinc 0.45 mg/L)	MHA with Zn supplement (3 mg/L)
*Meropenem	26.5 (1.8)	23.4 (1.0)
†Imipenem	22.0 (1.6)	19.4 (1.8)
‡Ceftazidime	20.9 (1.7)	21.6 (1.4)
§Piperacillin	21.7 (1.3)	22.0 (1.6)

* $p < 0.05$; † $p < 0.05$; ‡ $p > 0.05$; § $p > 0.05$.

either medium ($p > 0.05$). This effect was confirmed as specific for carbapenems only.

These observations have been explained in a recent study by Baxter and Lambert who showed that free zinc ions in aqueous solution hydrolyse imipenem and that the rate of hydrolysis depends on time and zinc concentration [5].

It is clear that, as for imipenem, the zinc concentration in MHA affects the activity of meropenem against *P. aeruginosa*. These observations may result in false reports of resistance to meropenem and imipenem and restrict the use of these antipseudomonal antibiotics in the treatment of *P. aeruginosa* infection.

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