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The Inhibitory Effect of Zinc on Imipenem and Meropenem Susceptibility of Pseudomonas aeruginosa

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Abstract: In this study we have showed that zinc ion has an inhibitory activity on imipenem and meropenem suspectibility of Pseudomonas aeruginosa. When zinc ion concentration of Mueller-Hinthon agar (MHA) media was gradually increased the zone diameters of imipenem and meropenem gradually decreased. So we concluded that zinc concentration in the media might effect imipenem and meropenem susceptibility of Pseudomonas aeruginosa and might indicate false resistance to imipenem and meropenem. Therefore the use of these antipseudomonal antibiotics in Pseudomonas infections may be restricted.

Key Words: P.aeruginosa, Zinc, Imipenem, Meropenem

Introduction

Imipenem and meropenem are broad-spectrum carbapenem antibiotics with activity against *Pseudomonas aeruginosa* (1-3). Beta-lactam rings of these antibiotics are resistant to hydrolysis by most beta-lactamases (4-5) and the activity of meropenem against most clinical isolates was comparable to imipenem (6). Thus these agents can be used in the treatment of infections caused by *Pseudomonas aeruginosa* isolates which are resistant to some groups of antibiotics (2-7). But there is some evidence that suggests false resistance of *Pseudomonas aeruginosa* to imipenem (8). Although this is partly attributed to the loss of drug activtiy during storage and it is also showed that the zinc concentration in the media would affect the imipenem susceptibility of *Pseudomonas aeruginosa* (9). Like this we also showed that the pencentage of pseudomonas aeruginosa isolates resistant to imipenem significantly increased in zinc-supplemented media (10).

In this study we wanted to observe the effects of zinc on imipenem and especially on meropenem against *Pseudomonas aeruginosa* by using the Kirby-Bauer method at various zinc concentrations.

Materials and Methods

In this study a total of 16 *Pseudomonas aeruginosa* isolates were studied. These isolates were obtained from the Microbiology Laboratory of the Medical Faculty of Dicle University. Fresh isolates were grown on MHA (Oxoid) for 24 hours at 37°C before use in each phase of the investigation. Control strain tested throughout the study was *Pseudomonas aeruginosa* ATCC 27853. Previously we had quantified the zinc concentration in the Oxoid MHA was 0.45 µg/ml (10). By adding zinc acetate in this media we increased zinc concentration of the media to 3 µg/ml. This concentration is very similar to 2.61 µg/ml which was quantified in BBL MHA (Lot no: H2DWFX) (9).

Sixteen isolates of *Pseudomonas aeruginosa* were adjusted to an optical density of 0.5 Mc Farland standart (10^8 cfu/ml) with sterile saline and then further diluted to achive a final bacterial concentration of 10^7 cfu/ml. The same bacterial isolates were grown on Oxoid MHA and zinc-supplemented Oxoid MHA. The antibiotic susceptibilities of *Pseudomonas aeruginosa* to imipenem (Lot no: Ch-B.40866, Oxoid) and meropenem (Lot no:45611, Oxoid) determined by Kirby-Bauer methods (11) on Oxoid MHA and zinc-supplemented Oxoid MHA after incubation of at 37°C for 24 hours. In all of these media, we obtained the zone diameters of imipenem and meropenem against *Pseudomonas aeruginosa.*

Results are expressed as mean ± SEM for sixteen experiments. If appropriate one-way paired or unpaired student's t test were used for statistical comparison. $P<0.05$ was considered to be significant.

Results

The results of the susceptibility testing of *Pseudomonas aeruginosa* isolates to imipenem and meropenem on Oxoid MHA and zinc-supplemented Oxoid MHA are shown in the table. All of these isolates used in this study were susceptible to imipenem and meropenem, however these isolates were more susceptible to meropenem than imipenem (P<0.005). The zone diameters of 16 isolates of *Pseudomonas aeruginosa* were 22±1.6 mm and 26.5±1.8 mm againts imipenem and meropenem respectively.

Table 1. The susceptibilityes of Pseudomonas aeruginosa isolates to imipenem and meropenem in two different amounts of zinc containing media (n:16). Each value represents mean±SEM of sixteen zone diameters.

Zinc Concentration in MHA (µg/ml) (a)	0.45 (b)	3
*Imipenem zone diatemers (mm)	22±1.6	19.4±1.8
**Meropenem zone diameters (mm)	26.5±1.8	23.4±1

* ab: P<0.05

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When zinc-supplemented Oxoid MHA was used the zone diameters of imipenem and meropenem against *Pseudomonas aeruginosa* isolates decreased (P<0.05). Observetaions for the control strain (*Pseudomonas aeruginosa* ATCC 27853) was similar to observation for the test strains (data not shown).

The susceptibilities of *Pseudomonas aeruginosa* isolates to imipenem and meropenem related with two different zinc concentrations are shown in the figure.

Discussion

The interest on the effect of zinc cation content in the media on imipenem susceptibility has been recently increased. And addition of zinc to Difco MHA (Lot no:780100) resulted in increases in MICs of imipenem for *Pseudomonas aeruginosa* but not in the MICs of some antibiotics (9). Similarly we observed that when zinc supplemented media was used the percentage of *Pseudomonas aeruginosa* isolates resistant to imipenem was significantly increased (10).

Imipenem and meropenem are carbapenem antibiotics and knowledge of the action of these beta-Lactam antibiotics is incomplete (12). These antibiotics pass through the outer membrane of gram-negative bacteriå via the water-filled porin channels (13) to reach their targest, penicillin binding proteins (14). Deletion or diminished production of these outer membrane proteins (porins) decreases outher membrane permeability of some gram-negative bacteria for diffusion of these antibiotics and decreases susceptibility to imipenem and meropenem (2, 15, 16). Although it is not clear at the

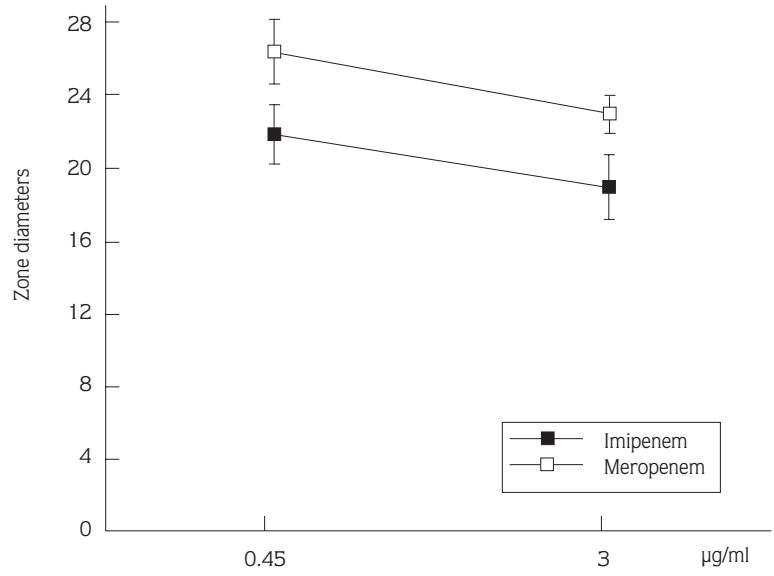

Figure 1. The susceptibility of P.aeruginosa isolates to imipenem and meropenem. Each point represents mean of sixteen zone diameters and SEM.

present time whether the porins are the only route of penetration of betalactam antibiotics, in particular for carbapenems it is probable that zinc would have to alter porins or decrease expression of multible porin proteins. If this is so we can explain why imipenem and meropenem zone diameters were inversely related to zinc concentration in the media. But some authors claim that the loss of porins alone should not cause resistance to a carbapenem (17).

The production of beta-lactamases had little influence on susceptibility to either meropenem or imipenem except the production of metalloenzymes capable of the hydrolyzing the carbanepenems, strains expessing high levels of these enzymes were resistant to both meropenem and imipenem (18). We know plasmid-encoded zinc-dependent beta-lactamase of *Pseudomonas aeruginosa* hydrolysis imipenem (19). But really we don't know whether zinc supplemented media affect the production of this type any enzyme or not.

Many antibiotics including beta-lactams can use porin pathway to enter in to the periplasm (20). Principal lethal target of carbapenem antibiotics in the cell are penicillin-binding proteins (21). Meropenem binds to penicillin-binding protein 3 and imipenem binds to penicillin-binding protein 2 (22). Zinc is known to affect penicilin binding protein-beta-lactam-interaction, but this effect has not been demonstrated in *Pseudomonas aeruginosa.* So the role of zinc ion on the carbapenems and penicillin-binding proteins of *Pseudomonas aeruginosa* isolates are not established.

Finally the real reasons for these observations are unknown and it is difficult to explain these results by any certainity. But we certainly showed that the zinc content in the Oxoid MHA does affect the activity of imipenem and of meropenem against *Pseudomonas aeruginosa.* The zone diameters of imipenem and meropenem against *Pseudomonas aeruginosa* isolates, obtained on Oxoid MHA with a high zinc content, are smaller: These observations may indicate false resistance to imipenem and meropenem and restrict the use of these

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