SHORT COMMUNICATION

In-vitro Susceptibility of *Burkholderia Pseudomallei* to Cefoperazone-Sulbactam Combination

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Summary

Melioidosis is endemic in Malaysia. Emerging resistance with new and current antimicrobial agents has underscored the need to look further for new antimicrobial agents for the treatment of melioidosis. Hence, we evaluated the in-vitro susceptibility of fifty locally isolated strains of *Burkholderia pseudomallei*, the causative agent of melioidosis to cefoperazone-sulbactam combination using the method of NCCLS. All the fifty strains tested were susceptible in-vitro to cefoperazone-sulbactam. The MIC_{90} of the organism for cefoperazone-sulbactam was 4mg/L. The results of our findings suggested that cefoperazone-sulbactam may be useful in the treatment of melioidosis.

Key Words: Melioidosis, Minimum inhibitory concentration, In-vitro susceptibility, Subactam-cefoperazone, *Burkholderia pseudomallei*

Introduction

Melioidosis is caused by *Burkholderia pseudomallei* and is endemic in Malaysia[1]. It is an opportunistic pathogen, and has the tendency to remain latent in infected individuals causing chronic infection or develop into septicaemic forms. Emerging resistance with current and new antimicrobial agents during therapy has underscored the need to look further for new antimicrobial agents for the treatment of melioidosis. With this, we evaluated the in-vitro antimicrobial susceptibility to cefoperazone-sulbactam combination in the ratio of 2:1 on 50 locally isolated strains of *B.pseudomallei* (30 from blood, 15 from pus, 2 from pleural fluid and 1 each from tissue, urine and sputum) isolated from 1992 to April 1996. Isolates were identified based on colonial morphology, gram stained appearance and biochemically. These strains were maintained on nutrient agar slope soon after isolation. We also assessed the antimicrobial activity of cefoperazone and sulbactam alone to determine the usefulness of sulbactam when used in the combination with cefoperazone. Cefoperazone and sulbactam powders were obtained from Pfizer. In addition, β-lactamase detection by the chromogenic cephalosporin method (Nitrocefin, OXOID) and drug disc diffusion test using the 75mcg cefoperazone disc and 75mcg cefoperazone-30mcg sulbactam disc (BBL,USA) were performed on all isolates.

The MICs were determined by the agar dilution method as recommended by NCCLS[2]. Serial two fold dilution of the antibiotics were made in Mueller-Hinton II agar (BBL,USA) in concentration ranging from 0.125-128mg/L for cefoperazone and 0.0625-64mg/L for sulbactam. MIC for cefoperazone-sulbactam combination was determined in the ratio of 2:1 with concentration ranging from 0.125/0.0625 to 64/32mg/L. A final inoculum of 10^4 cfu of the strains was applied onto freshly prepared antibiotic containing plates with a Denley Multipoint inoculator. *Pseudomonas aeruginosa* ATCC 27853 was used as control organism for cefoperazone and *Acinetobacter calcoaceticus* spp *anitratus* ATCC 43498 was included

Table I
MIC and geometric mean MIC (mg/L) of cefoperazone in the presence and absence of sulbactam for fifty clinical isolates of *Burkholderia pseudomallei*

Antimicrobial agents	Range	MIC_{50}	MIC_{90}	Geometric mean MIC
cefoperazone	8–32	16	32	16.0
sulbactam	2–8	4	4	3.7
cefoperazone-sulbactam	2–4	2	4	3.5

to monitor the sulbactam portion of the combination. The MIC was read as the lowest concentration of antibiotic that inhibited growth after overnight incubation at 36°C. Drug disc diffusion assay was performed according to the NCCLS guidelines[3]. Disc containing 75mcg cefoperazone and 75mcg cefoperazone-30mcg sulbactam were aseptically placed on the surface of the plates. Callipers were used to measure the diameter of zone inhibition after overnight incubation at 36°C.

All the fifty strains of *B.pseudomallei* tested produced β-lactamase. Forty-three strains were susceptible to cefoperazone (MIC 8-16mg/L) and all demonstrated zone sizes varying from 22-28mm. in diameter by the disc diffusion method. The remaining seven isolates demonstrated an MIC of 32mg/L, three gave intermediate results (20mm) and four were susceptible (21-23mm) with the disc diffusion method. All strains were susceptible when tested with 75mcg cefoperazone-30mcg sulbactam disc with zone diameter ranging from 31-36 mm. The results of the MIC tested with cefoperazone and sulbactam alone and in combination are summarised in Table I. The geometric mean MIC of cefoperazone and sulbactam tested alone with *B.pseudomallei* was 16.0 and 3.7 mg/L respectively and with cefoperazone-sulbactam combination was 3.5 mg/L. Reduction of MIC with cefoperazone-sulbactam combination by 1 $\log_2$ dilution was observed in 4 (8%), 2 $\log_2$ in 36 (72%) and 3 $\log_2$ in 10 (20%) cultures.

Effective treatment of melioidosis remains a problem. The organism is intrinsically resistant to many antibiotics. Treatment of melioidosis with conventional drugs such as doxycycline, tetracycline and chloramphenicol may be effective in mild infection, but they are not bactericidal. Combination of several agents have been recommended but they may cause considerable risks of toxicity on prolonged usage. Since the introduction and increased use of ceftazidime and co-amoxyclav in the treatment of melioidosis, resistance has emerged during treatment[4]. Cefoperazone had shown some activity against *B.pseudomallei* in previous studies[1] and based on data presented here, this activity was enhanced in the presence of sulbactam. When tested with cefoperazone and cefoperazone-sulbactam combination, the MIC_{90} values of the 50 strains tested were 32 and 4 mg/L, a reduction of MIC by eight fold. The seven cefoperazone resistant strains (MIC 32mg/L) were converted into the susceptible range. The results of our findings suggested that cefoperazone-sulbactam appeared promising and may be considered in the clinical management of melioidosis.

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