ORIGINAL ARTICLE

In Vitro Activity of Sulperazon Against Recent Isolates of Ceftazidime-Resistant Bacteria

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Summary

The *in vitro* activity of sulperazon (cefoperazone/sulbactam) was tested against 94 ceftazidime-resistant strains of bacteria isolated from mostly seriously ill patients in critical care units. *Acinetobacter baumanii, Pseudomonas aeruginosa* and *Klebsiella pneumoniae* made up 80% of the pathogens studied; 90% of the *Klebsiella* strains were producers of extended-spectrum β-lactamases (ESBL). The MIC_{90} of sulperazon for *Klebsiella* was 12mg/l (range 1.5-16 mg/l), indicating that this drug may be a useful alternative for the treatment of ceftazidime-resistant, ESBL-producing *Klebsiella.*

Key Words: Sulperazon, *In vitro* activity, ESBL-producing Klebsiella

Introduction

β-lactam antibiotics are frequently prescribed because they are broad-spectrum and relatively free from adverse side-effects. Unfortunately, in the last decade, their usefulness against bacterial pathogens have been greatly curtailed by the appearance of organisms resistant to penicillins and cephalosporins because of their ability to produce β-lactamases that can hydrolyse the amide bond in the β-lactam ring. β-lactamase producers now form over 90% of staphylococci[1] and 60% of *E.coli*[2] seen in hospitals. In the community, these enzyme producers are also found in increasing numbers among *Haemophilus influenzae, Moraxella catarrhalis*[3], and enteric bacteria[4]. Some newly emerged enzymes called extended-spectrum β-lactamases (ESBL) are able to degrade even broad-spectrum third generation cephalosporins like ceftazidime. These strains pose therapeutic problems because most of them are also resistant to aminoglycosides and fluoroquinolones[5].

In response to the β-lactamase challenge, the pharmaceutical industry has developed β-lactamase-resistant penicillins and cephalosporins as well as β-lactamase inhibitors like sulbactam, clavulanic acid and tazobactam. These inhibitors bind to active sites on the β-lactamase molecule, blocking its activity. Thus, when used together with β-lactam antibiotics, the inhibitors can prevent hydrolysis of the β-lactams and restore the usefulness of these drugs.

The inhibitor sulbactam has little intrinsic antibacterial activity except for strains of *Acinetobacter* and *Neisseria*, but it is active against the plasmid-encoded β-lactamase enzymes of most staphylococci and Gram-negative bacilli, including ESBL. In sulperazon, sulbactam is used in combination with cefoperazone, a third generation cephalosporin. Thus, it is potentially suitable for the treatment of moderate to severe hospital-acquired infections

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caused by Gram-negative nosocomiants including multi-resistant ESBL producers and *Pseudomonas aeruginosa.*

The objective of this study was to assess the *in vitro* activity of sulperazon against ceftazidime-resistant clinical isolates of Gram-negative bacilli including ESBL producers, and to compare this activity to that of 3 other antibiotics namely, amikacin, ciprofloxacin and imipenem.

Materials and Methods

Bacterial strains

The bacterial strains tested were isolates obtained from patients at the HUKM, a tertiary teaching hospital in Kuala Lumpur, between June and August 2000. These organisms were isolated from tracheal or nasopharyngeal aspirates, bronchoalveolar lavage, sputum, blood, CSF, urine, pus, catheter tips, conjunctiva, and tissues, from patients in intensive care and high dependency units as well as from medical, surgical, oncology and trauma wards, and outpatient clinics. Repeat isolates from the same patient were excluded.

Bacterial pathogens were isolated and identified by standard bacteriological procedures and tested for susceptibility to antibiotics by the agar disk diffusion test. Isolates showing ceftazidime resistance were selected for the determination of minimum inhibitory concentrations to ceftazidime, sulperazon, ciprofloxacin, amikacin and imipenem.

Determination of minimum inhibitory concentrations (MIC)

The MIC of antibiotics against ceftazidime-resistant strains were determined using the E-test (AB Biodisk, Solna, Sweden) according to the manufacturer's instructions. The interpretative breakpoints used were as recommended by the NCCLS except in the case of sulperazon. For this drug, since NCCLS guidelines were not available, the breakpoints used were supplied by Pfizer. The breakpoint MIC for sensitivity and resistance were 16 and 64mg/l respectively for sulperazon and amikacin; 1 and 4mg/l for ciprofloxacin and 4 and 16mg/l for imipenem.

The production of ESBL was also determined by the E-test. The presence of an ESBL producer was confirmed when there was a 16-fold or greater reduction in the MIC of ceftazidime in the presence of clavulanic acid.

Results

A total of 94 strains from 75 patients were studied. These bacteria included 38 strains of *Klebsiella pneumoniae*, 27 of *Acinetobacter baumanii*, 10 strains each of *Pseudomonas aeruginosa* and *Enterobacter species* and few strains each of *Stenotrophomonas maltophilia, Citrobacter species, Escherichia coli, Flavobacterium* and *Pseudomonas* species. Overall, 41 (44%) of the strains produced ESBL but 36 of these were *K. pneumoniae.* The MIC_{50} and MIC_{90} of sulperazon, amikacin, ciprofloxacin, imipenem and ceftazidime are compared in Table I.

Sulperazone was the only drug that was active against all 38 strains of ceftazidime-resistant *K. pneumoniae* (MIC range of 1.5-16mg/l). However, more than 50 % of *A. baumaniii* were resistant or had intermediate sensitivity to sulperazon although sulbactam has intrinsic activity against these bacteria. Similarly, against *Enterobacter sp.* and *P. aeruginosa*, sulperazon inhibited fewer strains than amikacin, ciprofloxacin and imipenem. Overall, for the 94 strains of ceftazidime-resistant and multi-resistant Gram-negative bacilli, the percentage of strains susceptible to sulperazon, amikacin, ciprofloxacin and imipenem was 52%, 50%, 53% and 82% respectively.

Table I
Minimum Inhibitory Concentrations of Sulperazon, Amikacin, Ciprofloxacin, Imipenem and Ceftazidime for 94 Clinical Isolates of Gram-negative Bacteria

Pathogen (No. ESBL+ ve / No. tested)	Antibiotic	MIC_{50} (mg/l)	MIC_{90} (mg/l)	MIC range (mg/l)
A. baumanii (0/27)	Sulperazon	32.0	128.0	2.0 - >256.0
	Amikacin	4.0	96.0	1.0 - 256.0
	Ciprofloxacin	>32.0	>32.0	0.19 - >32.0
	Imipenem	1.5	>32.0	0.38 - >32.0
	ceftazidime	>256.0	>256.0	8 - >256.0
Enterobacter sp. (3/10)	Sulperazon	12.0	>256.0	2.0 - >256.0
	Amikacin	2.0	8.0	0.75 - >256.0
	Ciprofloxacin	0.38	0.38	0.25 - 0.5
	Imipenem	0.38	0.38	0.25 - 0.5
	ceftazidime	128.0	>256.0	32.0 - >256.0
K. pneumoniae (36/38)	Sulperazon	6.0	12.0	1.5 - 16.0
	Amikacin	24.0	32.0	0.75 - 256.0
	Ciprofloxacin	0.5	3.0	0.008 - >32.0
	Imipenem	0.25	0.25	0.19 - >32.0
	Ceftazidime	>256.0	>256.0	3* - >256
P. aeruginosa (0/10)	Sulperazon	48.0	256.0	12.0 - 256.0
	Amikacin	12.0	>256.0	4.0 - >256.0
	Ciprofloxacin	0.19	6.0	0.125 - >32.0
	Imipenem	3.0	4.0	2.0 - 16.0
	Ceftazidime	128.0	>256.0	48.0 - >256.0
All strains (41/94)	Sulperazon	12.0	>256.0	1.5 - >256.0
	Amikacin	12.0	256.0	0.75 - 256.0
	Ciprofloxacin	0.5	>32.0	0.008 - >32.0
	Imipenem	0.38	>32	0.19->32.0
	ceftazidime	>256	>256.0	3*- >256.0

**1 strain of K. pneumoniae had a ceftazidime MIC of 3 mg/l but was found to be an ESBL producer*

Discussion

Antibiotics are life saving drugs for patients with serious sepsis. Early treatment with an appropriate drug is essential to decrease mortality. Empirical treatment should be based on local antimicrobial susceptibility patterns. Most multi-drug resistant strains are found in hospitalized patients compromised by debilitating illnesses, invasive procedures, and immunosuppressive therapy. The bacteria tested in this study were selected ceftazidime-resistant strains. Hence, the results obtained reflect the susceptibility patterns of multi-resistant bacteria affecting seriously ill hospital in-patients, many of whom were on mechanical ventilation and multiple-antibiotic treatment. As this type of patients are usually colonized by resistant bacteria, when infection is suspected, empirical therapy should be with antimicrobials that are broad-spectrum and effective against common multi-drug resistant bacteria.

ESBL-producing *K. pneumoniae* have become endemic in many hospital settings and have caused outbreaks of infection among hospitalized patients[6]. The genes encoding ESBL production are located on transmissible resistance plasmids that often carry other antibiotic resistance determinants. Bacteria with plasmids encoding multiple resistance determinants can be inadvertently selected out by the use of antibiotics to which the bacterial host is resistant. Hence, wherever possible, the choice of an antibiotic for therapy should be based on *in vitro* antibiotic susceptibility testing of the pathogen. Ceftazidime resistance is a marker for ESBL production but can also occur as a result of other resistance mechanisms. Among the *K. pneumoniae* in this study, 95% of the ceftazidime resistance was due to ESBL productrion, whereas, only 3 of the 10 *Enterobacter* and none of the *Acinetobacter* and *P. aeruginosa* strains were ESBL producers. In these bacteria in which ceftazidime resistance may be due to reduced outer membrane permeability, sulperazone was less effective than imipenem which has the ability to penetrate bacterial outer membranes rapidly. However, against ESBL-producers sulperazon was as effective as imipenem.

In conclusion, the results from this study showed that sulperazon should be a suitable alternative to imipenem for the treatment of infections caused by ESBL producing *K. pneumoniae.* As empirical therapy for suspected infection by multi-resistant Gram-negative bacilli, it should be as effective as amikacin and ciprofloxacin but with the advantages of being free from aminoglycoside toxicity and not being associated with the rapid emergence of resistance as occurs with ciprofloxacin among *P. aeruginosa.*

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