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Original Article

The impact of inoculum size on the activity of cefoperazone-sulbactam against multidrug resistant organisms

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Inoculum size

Abstract *Objectives:* This study aims to assess the in vitro activity of cefoperazone alone and different cefoperazone-sulbactam ratios against different inoculum sizes of multidrug resistant organisms.
Methods: Minimum inhibitory concentrations (MICs) of cefoperazone, cefoperazone-sulbactam at fixed ratio of 1:1 and 2:1 against a normal inoculum size of 5×10^5 CFU/ml and a high inoculum size of 5×10^7 CFU/ml were measured.
Results: Each 33 isolates of extended-spectrum β-lactamases (ESBL)-producing *Escherichia coli*, ESBL-producing *Klebsiella pneumoniae*, carbapenem-resistant *E. coli*, and carbapenem-resistant *Pseudomonas aeruginosa* and a total of 122 isolates of carbapenem-resistant *Acinetobacter baumannii* were collected. After the addition of sulbactam at a 1:1 ratio, most MIC_{50} and MIC_{90} values decreased. Cefoperazone-sulbactam at a 1:1 ratio had a higher susceptibility rate against ESBL-producing *E. coli*, carbapenem-resistant *E.* coli, and carbapenem-resistant *A. baumannii* than cefoperazone-sulbactam at a 2:1 ratio (all $P < 0.05$). For ESBL-producing *E. coli*, the susceptibility rate of cefoperazone-sulbactam at ratios of (1:1) and (2:1) decreased from 97.0 to 87.9% and 90.9 to 60.6%, for normal to high inoculum, respectively. For ESBL-producing *K. pneumoniae*, both susceptibility rate of cefoperazone-sulbactam at ratios of (1:1) and (2:1) decreased from 75.8%, and 63.6% at normal inoculum to 51.5% and 42.4% at high inoculum.

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Conclusions: Cefoperazone-sulbactam at a 1:1 ratio has greater in vitro activity against most multidrug resistant organisms than cefoperazone-sulbactam at a 2:1 ratio. Such combinations were not influenced by the inoculum size of ESBL-producing *E. coli and K. pneumoniae* and could be a therapeutic option for treating severe infections.

Introduction

Antibiotic resistance has become one of the world's most pressing health issues, and its emergence has weakened the ability of antibiotics to kill pathogenic organisms.[1–5] Although β-lactam antibiotics, including penicillin, cephalosporins, monobactams, and carbapenem, remain the major weapon against bacteria because of their broad-spectrum activity, clinical efficacy and safety,[6–9] widespread use of β-lactam antibiotics has also lead to the development of resistance to these antibiotics. The production of β-lactamases is the major mechanism that causes acquired β-lactam antibiotic resistance[10]; thus, the use of β-lactamases inhibitors in combination with β-lactam antibiotics, such as piperacillin-tazobactam, amoxicillin-clavulanate, and cefoperazone-sulbactam have been developed to overcome this mechanism.[11]

Gram-negative pathogens, including Enterobacteriaceae, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii*, are common pathogens that cause nosocomial infections, and these microorganisms carry the broad spectrum of the antibiotic resistance. For Enterobacteriaceae, the emergence of extended-spectrum β-lactamases (ESBL) among *Escherichia coli* and *Klebsiella pneumoniae* are the great threats to the management of infections. Most importantly, serious infections caused by ESBL-producing organisms result in higher mortality rates than non-ESBL producers, especially when the patients did not receive adequate antimicrobial therapy.[12–14] Recently, several studies showed the inoculum effects that the minimal inhibitory concentration (MIC) of an antibiotic would increase as well as the increasing number of the organisms in the inoculum.[15–19] This kind of laboratory phenomenon has been observed for several β-lactam antibiotics, such as piperacillin-tazobactam, amoxicillin-clavulanate, ceftriaxone, ertapenem and imipenem, against *E. coli* or *K. pneumoniae*.[15–19] In this study, the in vitro activity of cefoperazone-sulbactam against ESBL-producing *E. coli* and *K. pneumoniae* clinical isolates were investigated at an inoculum size of 5×10^5 CFU/ml and 5×10^7 CFU/ml. In addition, the in vitro activities of different cefoperazone-sulbactam compositions against ESBL-producing *E. coli*, ESBL-producing *K. pneumoniae*, carbapenem-resistant *E. coli*, carbapenem-resistant *P. aeruginosa*, and carbapenem-resistant *A. baumannii*, were also evaluated.

Materials and methods

Collection of clinical isolates

Thirty-three isolates of ESBL-producing *E. coli*, ESBL-producing *K. pneumoniae*, carbapenem-resistant *E. coli*, and carbapenem-resistant *P. aeruginosa* and 122 isolates of carbapenem-resistant *A. baumannii* were collected from sputum (n = 105), urine (n = 55), blood (n = 16), pus (n = 15), bile (n = 9), ascites (n = 5), and others (n = 8) from patients during the period of 2008–2015 by the department of bacteriology at Chi Mei Medical Center (Table 1). The isolates were stored at −80 °C in Protect Bacterial Preservers (Technical Service Consultants Limited, Heywood, UK) before use. ESBL phenotype among *E. coli* and *K. pneumoniae* isolates are confirmed by the method using the following four antimicrobial disks: cefotaxime, cefotaxime/clavulanic acid, ceftazidime and ceftazidime/clavulanic acid. An increase in the zone diameter by ≥5 mm for either antimicrobial agent tested in combination with clavulanic acid over when tested alone indicates that the isolate is an ESBL producer.[20] Carbapenem resistance is defined as resistant to imipenem, meropenem, doripenem, or ertapenem, and carbapenem-resistant phenotype among *P. aeruginosa* and *A. baumannii* are confirmed by the modified Hodge test. Species confirmation was performed by standard biochemical methods on a VITEK 2 automated system (bioMérieux, Marcy l'Etoile, France).

Table 1 The number of positive specimen for each bacterium.

Number of specimens	ESBL-producing *E. coli*	ESBL-producing *K. pneumoniae*	Carbapenem-resistant *E. coli*	Carbapenem-resistant *P. aeruginosa*	Carbapenem-resistant *A. baumannii*	Total
Blood	9	4	3	0	0	16
Urine	10	8	18	9	10	55
Sputum	4	14	6	22	100	146
Ascites	3	1	1	0	0	5
Bile	4	2	0	1	2	9
Pus	3	1	4	0	7	15
Others	0	3	1	1	3	8

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