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Original Article

Ceftaroline activity on certain respiratory tract and wound infection agents at the minimum inhibitory concentration level

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Abstract

Introduction: The aim of this study was to investigate the effectiveness of ceftaroline against agents frequently isolated from respiratory tract and wound infections.

Methodology: The study included a total of 250 strains isolated from various clinical specimens, among which were *Streptococcus pyogenes*, *Streptococcus agalactiae*, *Streptococcus dysagalactiae*, *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Moraxella catharralis*. The bacteria were identified using the matrix-assisted laser desorption/ionization time-of-flight method and conventional methods. The bacteria's antibiotic susceptibility was tested using appropriate broth microdilution. Mueller-Hinton broth with 4% lysed horse blood, Haemophilus test medium broth, and Mueller-Hinton broth were used. Ceftaroline fosamil results at the minimum inhibitory concentration (MIC) were evaluated using Clinical and Laboratory Standards Institute (CLSI) criteria. For quality assurance, *E. coli* ATCC 35218, *S. aureus* ATCC 29213, *S. aureus* ATCC 43300, *S. pneumoniae* ATCC 49619, *H. influenzae* ATCC 49766, *H. influenzae* ATCC 10211, and *H. influenzae* ATCC 49247 standard strains were used.

Results: According to CLSI criteria, resistance was not detected in any strains. Due to the absence of CLSI criteria for *M. catharralis*, the susceptibility state for this bacterium was not evaluated. The various strains' MIC_{50}–MIC_{90} values were as follows: for *S. pyogenes*, 0.015–0.06; for *S. agalactiae*, 0.03–0.125; for *S. dysagalactiae*, 0.03–0.06; for *S. pneumoniae*, 0.06–0.125; for *H. influenzae*, 0.015–0.125; and for *M. catharralis*, 0.5–1.

Conclusions: The results indicate that ceftaroline is quite effective against bacteria that are frequently isolated from respiratory tract and wound infections.

Key words: *S. pneumoniae*; *H. influenzae*; *M. catharralis*; streptococcus; ceftaroline; MIC.

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Introduction

Respiratory tract and wound infections are the most commonly encountered infections in the community. Community-acquired pneumonia is a major cause of morbidity and mortality throughout the world [1,2]. In addition to fatal respiratory tract infections, there are wound infections reported that are unresponsive to empiric treatment [3]. Complicated wound infections can require inpatient treatment and surgical intervention, and can frequently cause life-threatening conditions. Antibiotics continue to be important for treating infections of these kinds; hence, new treatment options must be developed to prevent emerging resistance [4]. Because of these considerations, there will continue to be studies to observe developing antibiotic resistance and to identify new and more efficient treatment options [2,3]. As part of a new generation of cephalosporins, ceftaroline fosamil is a broad-spectrum antibiotic approved by the United States Food and Drug Administration (FDA) for the treatment of respiratory tract and wound infections [5-7]. Our study aimed to investigate the effectiveness of the new generation of cephalosporins such as ceftaroline against frequently encountered respiratory tract and wound infection agents using the microdilution method.

Methodology

Bacteria isolation

The study included a total of 250 strains, including *Streptococcus pyogenes* (n = 49), *S. agalactiae* (n = 98), *S. dysagalactiae* (n = 12), *S. pneumoniae* (n = 29), *Haemophilus influenzae* (n = 45), and *Moraxella catharralis* (n = 17), isolated from various clinical

samples between 2012 July and 2014 July (Table 1). Only one bacterial strain was studied from any given patient, and repeating strains were skipped. Bacteria were identified using conventional tests (the pyrrolidonyl arylamidase [PYR] test, the Christie Atkins Munch-Petersen (CAMP) test, and satellite and hockey puck test) as well as matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) (BioMérieux, Marcy L'Etoile, France). MALDI-TOF MS fingerprinting is a method used for the classification and identification of microorganisms with applications in clinical diagnostics. It allows the analysis of molecules with higher masses, while mass spectrometry detects the mass-to-charge ratio of a bioanalyte and provides bacterial spectra within minutes. A database of known organisms is used to match the isolate under investigation, providing a matching score based on identified masses and their intensity correlation. As noted by De Carolis *et al*., this technology is rapid, robust, customizable pursuant to the needs of the laboratory, more cost effective than current phenotypic testing methods despite the initial cost of the instrument, and, perhaps most importantly, easy to use [8].

Susceptibility study

For *S. pneumoniae* and the A, B, and C groups of β-hemolytic streptococci, a Mueller-Hinton broth with 4% lysed horse blood was used. For *H. influenzae,* a Haemophilus test medium was made by mixing Mueller-Hinton broth powder and yeast extract with 4% lysed horse blood, while for *M. catharralis*, a Mueller-Hinton broth was used. A ceftaroline (Zinforo, AstraZeneca, London, UK) 4,096 µg/mL stock solution was made by dissolving powder in dimethyl sulfoxide (DMSO). Standard 96-well microdilution plates were used, with 100 µL of broth placed in each well and serial dilutions made until the final dilution was achieved. The antibiotic dilution ranges in the wells were 0.003–2 µg/mL. In every plate, positive- and negative-control wells were used. For the inoculum, suspensions were prepared with a turbidity of 0.5 colony-forming units (CFUs)/mL using fresh cultures. All except the negative control wells were inoculated with 100 µL for each 1/100 dilution. The streptococci strains and *H. influenza* were incubated in 5% carbon dioxide (CO_2), and *M. catharralis* was incubated in an ambient atmosphere incubator, all for 24 hours. The respective strains' minimum inhibitory concentration (MIC) values were established using 2013 Clinical and Laboratory Standards Institute (CLSI) breakpoint values, and both MIC_{50} and MIC_{90} values were calculated [9].

For quality control purposes, *E. coli* ATCC 35218, *S. aureus* ATCC 29213, *S. aureus* ATCC 43300, *S. pneumoniae* ATCC 49619, *H. influenzae* ATCC 49766, *H. influenzae* ATCC 10211, and *H. influenzae* ATCC 49247 strains were used.

Results

The strains included in the study comprised 49 *S. pyogenes*, 98 *S. agalactiae*, 29 *S. pneumoniae*, 12 *S. dysagalactiae*, 45 *H. influenzae*, and 17 *M. catharralis*. The strains studied and the samples from which they were isolated are shown in Table 1.

Evaluation according to 2013 CLSI breakpoint values showed that *S. pyogenes*, *S. agalactiae*, *S. dysagalactiae*, *S. pneumoniae*, and *H. influenzae* isolates were susceptible to ceftaroline. MIC ranges for the strains were as follows: for *S. pyogenes*, 0.003–0.5 µg/mL; for *S. agalactiae*, 0.007–0.5 µg/mL; for *S. dysagalactiae*, 0.007–0.25 µg/mL; for *S. pneumoniae*, 0.003–0.25 µg/mL; for *H. influenzae*, 0.003–0.25 µg/mL; and for *M. catharralis*, 0.125–2 µg/mL. MIC_{50} and MIC_{90} values detected were as follows: for *S. pyogenes*, 0.015–0.06 µg/mL; for *S. agalactiae*, 0.03–0.125 µg/mL; for *S. dysagalactiae*, 0.03–0.06 µg/mL; for *S. pneumoniae*, 0.06–0.125 µg/mL; for *H. influenzae*, 0.015–0.125 µg/mL; and for *M. catharralis*, 0.5–1.0 µg/mL. Results are shown in Table 2.

Table 1. Strain types and sample distribution

Bacteria		Throat smear n = 36 (14.4%)	Wound smear n = 25 (10%)	Urine n = 111 (44.4%)	Blood n = 13 (5.2%)	**RTS** n = 63 (25.2%)	CSF n = 2 (0.8%)
S. pyogenes	n = 49	34	7	1	1	3	
S. agalactiae	n = 98		5	91	2		
S. dysagalactiae	n = 12	2	8	3	2		
S. pneumoniae	n = 29		3		7	17	2
H. influenzae	n = 45		2			43	
M. catharralis	n = 17			16	1		

RTS: respiratory tract samples; CSF: cerebrospinal fluid

Table 2. Strains' ceftaroline minimum inhibitory concentration (MIC) distribution

Bacteria		≤ 0.003	0.007	0.015	0.03	0.125	0.06	0.125	0.25	0.5	1	2	$MİC_{50}$	$MİC_{90}$
S. pyogenes	n = 49	8	9	13	9	2	7	2		1			0.015	0.06
S. agalactiae	n = 98		10	38	12	16	15	16	5	2			0.03	0.125
S. dysagalactiae	n = 12		5	1	2		3		1				0.03	0.06
S. pneumoniae	n = 29	6	1	4	3	10	4	10	1				0.06	0.125
H. influenzae	n = 45	2	9	12	8	7	3	7	4				0.015	0.125
M. catharralis	n = 17					1		1	4	8	2	2	0.5	1

Discussion

Various studies have documented emerging antibiotic resistance over the course of time among respiratory tract and wound infection agents. This developing resistance to first-line antibiotics has led us to seek different alternatives for treatment. Although no studies of penicillin-resistant pneumococcus cases were published for many years, there are numerous recent studies noting increasing resistance [10-12]. In addition, the presence of a penicillin allergy in 10% of the community, as well as reported resistance to substitute antibiotics such as macrolides for the treatment of community-acquired pneumonia, have caused a demand for the development and utilization of new alternatives [1].

Crespo-Ortiz *et al*. reported in a 17-year retrospective study that resistance to *S. agalactiae* isolates was emerging in Colombia [13]. While no resistance to cefaclor and cefuroxime was noted with *M. catharralis* isolates during 1993–1994, Hsu *et al*. reported 8.3% and 1.3% resistance, respectively, in their study. In addition, ampicillin resistance was detected in all β-lactamase-producing *M. catharralis* isolates [12]. Farrell *et al*. and Flamm *et al.* reported that penicillin resistance was emerging in *S. pneumoniae* isolates [14,15]. A study by Rennie *et al.* pointed to a 9.1% resistance to amoxicillin clavulanic acid in *H. influenzae* isolates [16]. Increasing erythromycin resistance to *S. pyogenes* was reported in 1970 in Japan, and the same condition was observed in European Union countries. Erythromycin, clarithromycin, and azithromycin resistance in the A, B, and C groups of β-hemolytic streptococci was encountered in a multicenter study performed in the United States [17].

As part of a new generation of cephalosporins, ceftaroline fosamil is a broad-spectrum antibiotic approved by the FDA for the treatment of respiratory tract and wound infections [5-7]. Ceftaroline's effectiveness is related to its high binding affinity to modified penicillin-binding proteins as compared with other β-lactam antibiotics. Furthermore, ceftaroline is reported as being effective *in vitro* for bacteremia patients with penicillin-resistant *S. pneumoniae* [7]. Since its approval in 2010, few clinical data is available about the efficacy of ceftaroline fosamil.

Fenoll *et al.* described the development of resistance to penicillin, amoxicillin, and cefotaxime in *S. pneumoniae* isolates, but were not able to evaluate ceftaroline's effectiveness, although they derived an MIC_{90} value of 0.25 μg/mL [18]. A study performed in the United States showed a ceftaroline MIC range between 0.004 μg/mL and 0.25 μg/mL for both *S. pyogenes* and *S. pneumoniae* isolates. Clark *et al.* at the Hershey Medical Center demonstrated a 0.06–8 μg/mL MIC range for *H. influenzae* and *M. catharralis* isolates [19]. In their study, Flamm *et al.* reported that ceftaroline at 0.5 μg/mL inhibits all *S. pneumoniae* isolates, with MIC values for *H. influenzae* and *M. catharralis* of MIC_{50}/MIC_{90} ≤ 0.015/0.03 μg/mL and MIC_{50}/MIC_{90} 0.06/0.12 μg/mL, respectively [14]. Pfaller *et al*. investigated 5,530 isolates in their study and showed that ceftaroline is potent and effective against *S. pneumoniae* (MIC_{50} 0.01 μg/mL, MIC_{90} 0.12 μg/mL), *H. influenzae* (MIC_{50} 0.008 μg/mL, MIC_{90} 0.015 μg/mL), *M. catharralis* (MIC_{50} 0.06 μg/mL, MIC_{90} 0.12 μg/mL) isolates. MIC ranges for *S. pneumoniae* were ≤ 0.008–0.5 μg/mL, for *H. influenzae* ≤ 0.008–0.5 μg/mL, and for *M. catharralis* ≤ 0.008–1 μg/mL. They reported ceftaroline to be the safest antibiotic deployable for empiric treatment of serious community-acquired pneumonia, especially resistant and refractory *S. pneumoniae* strains [20]. Farrell *et al.* investigated the ceftaroline susceptibility of a total of 460 *S. pyogenes* and *S. agalactiae* isolates and identified the MIC ranges as ≤ 0.008–0.015 μg/mL and ≤ 0.008–0.03 μg/mL, respectively [21].

Our study included bacterial agents that are frequently isolated from respiratory tract and wound infections. These strains were identified using the latest technology in microbiological identification, particularly the MALDI-TOF method. We had an opportunity to rapidly identify and investigate the

ceftaroline susceptibility of *S. dysagalactiae* strains, which are difficult to identify via phenotypic methods. Unfortunately, the number of these strains was small. MIC ranges and MIC_{50} and MIC_{90} values for streptococcus and *H. influenzae* strains used in our study were similar to those found in other studies. This result gives rise to the observation that ceftaroline is effective against strains isolated from different regions.

The search for new antibiotics for treatment has come about because of the emergence of β-lactamase-producing *M. catharralis* strains. The MIC range of *M. catharralis* isolates that were included in our study was between 0.125 and 2 µg/mL. These values are slightly higher than those found in other studies. Our data will perhaps be an example to other studies that may use an increased number of strains. On the other hand, the fact that current CLSI standards offer no evaluation data for MIC values makes research in this area difficult.

Conclusions

Our study showed that ceftaroline is quite effective against multiple and diverse bacterial isolates from different clinical samples of respiratory tract and wound infections. We recommend that ceftaroline could be a reasonable alternative for the treatment of these infections.

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