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Methodological agreement on the in vitro activity of ceftaroline against cefotaxime-susceptible and -resistant pneumococci

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ABSTRACT

Ceftaroline reportedly has lower minimum inhibitory concentrations (MICs) than established cephalosporins for *Streptococcus pneumoniae*. We further evaluated this activity using 155 pneumococci chosen by serotype and cefotaxime MIC. MICs were determined by agar dilution on Mueller–Hinton agar and Iso-Sensitest agar and by Etest. Inhibition zones were measured for 5 μg and 30 μg ceftaroline discs using both CLSI/EUCAST and BSAC methodology. Ceftaroline was more active than cefotaxime, with MICs 2–8-fold lower for isolates with cefotaxime MICs of ≤1 mg/L and mostly in the range 0.125–0.5 mg/L for those with cefotaxime MICs of 2 mg/L to ≥16 mg/L. Twelve isolates belonging to serotypes 14 (n = 2), 19A (n = 6) and 19F (n = 4) were ceftaroline-resistant, with MICs of 0.5–1 mg/L. Essential agreement between MIC methods was excellent, with values on Iso-Sensitest agar and Mueller–Hinton agar identical ±1 doubling dilution in all cases, and with 154/155 values identical ±1 doubling dilution between agar dilution and Etest. Nevertheless, 5/11 isolates with agar dilution MICs of 0.5 mg/L (i.e. just resistant) 'had' MICs of 0.25 mg/L (just susceptible) by Etest. Inhibition zones also correlated with MICs, but discrimination around the breakpoint MICs was poor irrespective of method and disc type. In summary, the results confirm the good activity of ceftaroline against pneumococci, but susceptibility testing will present challenges in routine laboratories, with discs poorly discriminatory and with Etest prone to give susceptible results for isolates with MICs one doubling dilution above the breakpoint.

1. Introduction

Ceftaroline reportedly has four- to eight-fold superior minimum inhibitory concentrations (MICs) to cefotaxime and ceftriaxone for pneumococci, including for strains with reduced susceptibility to penicillin and cephalosporins [1,2]. This behaviour correlates with strong affinity for the penicillin-binding proteins of pneumococci, even when these are modified by mutation and mosaic formation [3].

In this study, this activity was further characterised, testing ceftaroline by agar dilution, Etest and disc diffusion against a large panel of previously serotyped UK pneumococci, graded by cefotaxime MICs, seeking also to define agreement between methods.

2. Materials and methods

2.1. Streptococcus pneumoniae isolates

Isolates (n = 155) were selected from among recent submissions to Public Health England for reference investigation and were chosen to represent a wide range of cefotaxime MICs and serotypes (Table 1). Cefotaxime-resistant organisms, inevitably, were concentrated into a narrower range of serotypes, predominantly 6B, 9V, 14, 19A and 19F.

2.2. Antibiotics and susceptibility tests

Ceftaroline powder was from AstraZeneca (Macclesfield, UK). MICs were measured both by the British Society for Antimicrobial Chemotherapy (BSAC) agar dilution method [4] [i.e. incubated for 18 h in a 5% CO_2 atmosphere on Iso-Sensitest agar from Oxoid Ltd. (Basingstoke, UK) supplemented with 5% equine blood] and by an agar dilution method corresponding as closely as possible to Clinical and Laboratory Standards Institute (CLSI) broth microdilution criteria [5] using Mueller–Hinton agar (Oxoid Ltd.) supplemented with 5% ovine blood incubated in air for 20 h.

Ceftaroline E-tests (bioMérieux, Lyos, France) were used in accordance with the manufacturer's directions, i.e. on Mueller–Hinton agar supplemented with 5% ovine blood and incubated for 20 h in air. Disc diffusion tests were performed using 5 μg (Oxoid Ltd.) and 30 μg (Mast Group Ltd., Bootle, UK) discs. Both BSAC [4] and CLSI/European Committee on Antimicrobial Susceptibility Testing (EUCAST) [6] methodologies were followed, using Iso-Sensitest agar with 5% equine blood in a 5%

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Table 1
Serotype distribution of isolates in relation to cefotaxime minimum inhibitory concentration (MIC).

Serotype	No. of isolates with cefotaxime MIC (mg/L)								
	<0.06	0.12	0.25	0.5	1	2	4	8	>16
6						1			
6B							3		
6C	1	1		1	1				
7B		1	1						
7C	1	1							
8	1			1					
9				1	1				
9A				1	1				
9N	1				1				
9V					1	1	2		
10A	1		1						
11A	1	1		1	1	1			
12F	1								
13	1	1		1					
14						1	3	1	
15A		1	1	1	1	1			
15B	1	1							
16F	1	1				2			
17F	1								
18F				1					
19				1	1	2		2	
19A	4	2	2	6	5	7	8	3	1
19F						1	8	7	1
20	1								
21		1	1						
22F	1								
23						1			
23B	1								
23F						1	1		
24F	1								
28A	1								
31	1								
33F	1	1			1				
34	1								
35A				1					
35B	5	2	5	4	4	1			
35C					1				
35F	1								
37	1								

CO_2 atmosphere and Mueller–Hinton agar with 5% ovine blood in air, respectively. Zones were measured in three directions and were averaged.

3. Results

3.1. Relationship of ceftaroline and previous cefotaxime minimum inhibitory concentrations

MICs of ceftaroline rose in parallel with the previously determined cefotaxime MICs up to a cefotaxime MIC of ca. 1 mg/L (Table 2), typically with the ceftaroline MIC two- to eight-fold below that of cefotaxime. For isolates with cefotaxime MICs > 1 mg/L, the ceftaroline MICs mostly were in the range of 0.125–0.5 mg/L, and the relationship between values for the two agents weakened. In total, 39 isolates scored as intermediate (MIC = 1–2 mg/L) and 40 as resistant (MIC > 2 mg/L) to cefotaxime according to EUCAST criteria, but only 12 were counted non-susceptible to ceftaroline on Mueller–Hinton agar at the single EUCAST and CLSI >0.25 mg/L breakpoint, whilst 11 were non-susceptible on Iso-Sensitest agar. Thirty-nine serotypes were represented in the whole collection, but the ceftaroline-non-susceptible isolates all belonged to serotypes 14 ($n = 2$), 19A ($n = 6$) and 19F ($n = 4$).

Table 2
Relationship of ceftaroline and cefotaxime minimum inhibitory concentrations (MICs) for *Streptococcus pneumoniae*.

MIC of ceftaroline	Previous cefotaxime MIC (mg/L) by BSAC agar dilution methodology[a]								
	<0.06	0.125	0.25	0.5	1	2	4	8	>16
On Mueller–Hinton agar									
<0.008	30	1							
0.015		1							
0.03		5	1						
0.06		8	5	4					
0.125			5	16	18	11	2		
0.25					1	9	18	6	2
0.5							5	6	
1								1	
On Iso-Sensitest agar									
<0.004	4								
0.008	26	1							
0.015		1							
0.03		5	1						
0.06		8	8	2					
0.125			2	18	19	11	4	1	
0.25						9	15	8	1
0.5							6	4	1

BSAC, British Society for Antimicrobial Chemotherapy.
[a] Dark grey shading indicates susceptible to cefotaxime by European Committee on Antimicrobial Susceptibility Testing (EUCAST) criteria; light grey shading indicates intermediate to cefotaxime by EUCAST criteria unshaded indicates resistant to cefotaxime on.EUCAST criteria.

Table 3
Agreement of ceftaroline minimum inhibitory concentrations (MICs) between methods.

MIC of ceftaroline	MIC of ceftaroline (mg/L) on Mueller-Hinton agar [a]							
	0.008	0.015	0.03	0.06	0.125	0.25	0.5	1
On Iso-Sensitest agar								
0.004	4							
0.008	27							
0.015			1					
0.03		1	4	1				
0.06			1	14	3			
0.125				2	47	6		
0.25					2	28	3	
0.5						2	8	1
1								
By Etest								
0.008	31		1					
0.015		1						
0.03			4	2				
0.06			1	12	1			
0.125				3	44	1		
0.25					7	34	5	
0.5						1	6	
1								1

[a] Dark grey shading indicates susceptible to ceftaroline by European Committee on Antimicrobial Susceptibility Testing (EUCAST) criteria; unshaded indicates resistant.