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Molecular characterization of penicillin-binding-protein-3 in clinical isolates of *Haemophilus influenzae* with reduced susceptibility to cefotaxime, taken from the British Society for Antimicrobial Chemotherapy respiratory resistance surveillance study 2004-2008.

20th ECCMID 10-13th April 2010
Vienna, Austria

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Background

Haemophilus influenzae is an invasive pathogen associated with severe and often fatal cases of respiratory disease (1).

Cephalosporin antibiotics such as cefuroxime and cefotaxime are the drugs of choice for the treatment of *Haemophilus* infections. Cephalosporin resistance began to emerge in the last decade and the molecular mechanisms underlying the resistance have been identified (2). Resistance is mediated by two known mechanisms; one is the production of a β-lactamase and the other the result of mutations in the β-lactam targets; the penicillin binding proteins.

For non-β-lactamase producing isolates of *H. influenzae*, resistance to cefuroxime is well documented (2, 3), yet reduced susceptibility to cefotaxime is extremely rare. Penicillin binding protein-3 (PBP3) encoded by the *ftsI* gene plays an integral role in peptidoglycan synthesis. Kishii *et al* have demonstrated that mutations in the *ftsI* are the most important for the development of resistance to β-lactams in non-β-lactamase strains (4).

The British Society for Antimicrobial Chemotherapy (BSAC) respiratory resistance surveillance study monitors resistance in lower respiratory infections in the United Kingdom and Ireland.

The objective of this study was to identify the mechanism of cefotaxime non-susceptibility in three non-β-lactamase producing strains of *H. influenzae* collected from 2004/05-2007/08.

Methods

Minimum inhibitory concentration (MIC) was determined for 3740 clinical isolates of *H. influenzae* using the BSAC agar dilution susceptibility testing method (5). BSAC breakpoints were used to determine cefotaxime susceptibility, ≤0.12 mg/L for susceptible and >0.12 mg/L for resistant (5).

Methods cont.

To determine the mechanism of cefotaxime non-susceptibility, *ftsI* primers previously published by Straker *et al* were used to amplify by PCR part of the *ftsI* gene encoding PBP3 (2). Sequencing was performed on an ABI Prism 3100 Genetic Analyzer (Applied Biosystems) and analyzed using the DNA STAR analysis program (DNASTAR, Madison, Wis.).

Results

Of 3740 clinical *H. influenzae* isolates, 3204 (85.6%) were β-lactamase negative. Of these, 3116 (97.2%) had cefotaxime MICs of ≤0.06 mg/L, 66 (2.1%) had MICs of 0.12 mg/L and 22 (0.7%) were resistant to cefotaxime with MICs of ≥0.25 mg/L. Of these resistant isolates two (HI040610 and HI062032) had a cefotaxime MIC of 0.5 mg/L and one (HI072003) had a cefotaxime MIC of 1 mg/L.

The table below shows the amino acid substitutions found in the PBP3 of the three isolates with MIC of 0.5 and 1 mg/L, with two highly susceptible isolates and two reference strains for comparison.

Isolate	Cefotaxime MIC (mg/L)	PBP3 Amino Acid positions					
		350	490	502	511	517	526
HI072031	0.008	D	G	A	V	R	N
HI072049	0.008	D	G	A	V	R	N
ATCC 49247	0.015	D	G	A	V	R	**K**
NCTC 11931	0.12	**N**	G	A	V	R	N
HI040610	0.5	D	**E**	**V**	V	R	**K**
HI062032	0.5	D	G	A	V	**H**	N
HI072003	1	D	G	A	**A**	**H**	N

Discussion

Sequencing of the cefotaxime resistant isolate HI072003 revealed the substitutions V511A and R517H in PBP3. It has been previously reported that the substitutions V511A and R517H are common among isolates of *H. influenzae* with reduced susceptibility to cefuroxime (2, 3).

H. influenzae recombinant strains carrying the R517H substitution in PBP3 have a 4-16 fold increase in cefotaxime MIC compared to a susceptible laboratory strain. In addition the accumulation of mutations within PBP3 can cause an increase in cephalosporin MICs (6).

We propose that the substitution of valine (V) and arginine (R) at amino acid positions 511 and 517 with the larger branched chain amino acids alanine (A) and histidine (H) could result in hindrance between the binding site of β-lactam antimicrobials and penicillin-binding proteins, conferring cefotaxime resistance.

Conclusions

We have shown that a combination of one or more mutations within the region adjacent to the PBP3 (between residue positions 490 and 517) are associated with higher cefotaxime MICs (0.5 mg/L or 1 mg/L).

References

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Acknowledgements

We would like to acknowledge and thank the following for their support and sponsorship of the study: Astellas, Cerexa, Johnson & Johnson, Merck Sharp & Dohme, Novartis, Pfizer, Theravance, Wyeth and the BSAC.

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