Beta-lactamase Production in Recent Clinical Isolates of *Haemophilus influenzae* and their Susceptibility to Cefaclor and Other Antimicrobial Agents

S. M. H. Qadri, G. C. Lee, M. E. Ellis


The ability of 383 recent clinical isolates of *Haemophilus influenzae* to produce beta-lactamase was detected by using cefinase discs. It was found that 8% of the isolates elaborate this enzyme. Since cefaclor supposedly is not inactivated by beta-lactamases of *H. influenzae*, we studied the *in vitro* activity of this cephalosporin and compared it with other commonly used antimicrobial agents against isolates of *H. influenzae* from 383 patients at a tertiary care referral hospital in Riyadh, Saudi Arabia. Cefaclor was found to be most active, inhibiting 98% of the isolates tested, followed by amoxycillin/clavulanic acid and gentamicin (95%), chloramphenicol (93%), cephalothin (90%), ampicillin (87%), tetracycline (77%) trimethoprim-sulphamethoxazole (65%) and erythromycin (47%).

*Haemophilus influenzae* primarily infects the upper respiratory tract of man and may constitute the normal flora of the nasopharynx in up to 80% of healthy people. This asymptomatic nasopharyngeal infection sometimes develops into symptomatic disease involving sinuses, middle ear, lungs, bronchi, epiglottitis, pericardium, joints, blood stream and meninges. Ampicillin has been the drug of choice for treating many of these infections because of its excellent *in vitro* activity, few side effects and oral dosing.\(^1\)\(^2\) However, with the emergence of ampicillin resistance during the 1970s, other drugs like chloramphenicol, erythromycin, tetracycline and trimethoprim-sulphamethoxazole have been used. Since many of them are associated with serious side-effects, the search continues for a safer yet more effective drug. Cefaclor is such an oral cephalosporin with a chemical structure of 7-[D-(amino-phenylacetyl) amino]-3-chloro-3-cephem-4-carboxylic acid.\(^3\) In this paper we describe the *in vitro* activity of this cephalosporin and comparison with other commonly used antimicrobial agents against over 380 recent clinical isolates of *H. influenzae*.

Materials and Methods

Antimicrobials agents

Agar diffusion tests were performed using commercially prepared antibiotic discs (BBL, Cockeysville, Md.). Cefaclor (30 µg) discs were obtained from Eli Lilly Co. (Riyadh, Saudi Arabia). The antibiotic content of the
discs was 10 μg for ampicillin and gentamicin, 15 μg for erythromycin, 30 μg for cephalothin, chloramphenicol, and tetracycline, 1.25 μg, 23.75 μg for trimethoprim-sulphamethoxazole and 20/10 μg for amoxycillin/clavulanic acid. The disc contents used are those recommended by the National Committee for Clinical Laboratory Standards for which interpretive data have been established.

Bacteria

Isolates from clinical specimens of 383 patients were tested over a 6-month period at King Faisal Specialist Hospital and Research Centre, Riyadh, Saudi Arabia. The KFSH is a 550 bed tertiary care hospital which serves as a referral facility for the Kingdom of Saudi Arabia and the Middle East. Haemophilus influenzae were isolated from blood, cerebrospinal fluid or respiratory tract of 383 different patients and identified by conventional methods described in the Manual of Clinical Microbiology.

Detection of beta-lactamase activity

Commercially available cefinase discs, which were impregnated with the chromogenic cephalosporin nitrocefin (BBL, Cockeysville, MD, USA), were used for the rapid detection of beta-lactamase production by H. influenzae. When performed as per the manufacturer’s directions, the presence or absence of beta-lactamase production was established within 1–5 minutes.

Susceptibility testing

Antibiotic disc susceptibility testing was performed according to the procedure described by the National Committee for Clinical Laboratory Standards. Microorganisms were considered susceptible to cefaclor if the zone size was equal to or greater than 18 mm. Haemophilus test medium containing haematin and factor V & X supplement with a pH between 7.2 and 7.4 was used in the tests. Inoculum size was between 1–4 x 10^8 CFU/ml. The susceptibility plates were incubated for 18–24 h at 35°C in a CO₂ incubator. Inoculum size, and interpretive data used was same as described previously.

Results

The strains of H. influenzae isolated from clinical specimens of 383 patients were used to determine the beta-lactamase production. Each isolate came from a separate patient. Thirty-one of these isolates were found to be beta-lactamase producers and all of them were later found to be resistant to ampicillin. In vitro activity of cefaclor, cephalothin, ampicillin, amoxycillin/clavulanic acid, erythromycin, chloramphenicol, tetracycline, gentamicin and trimethoprim-sulphamethoxazole was performed against all the isolates, beta-lactamase producers as well as non-producers. Cefaclor was found to be most active, inhibiting 98% of the isolates, followed by amoxycillin/clavulanic acid, gentamicin, and chloramphenicol (Table 1). Erythromycin and trimethoprim-sulphamethoxazole were the least effective.

Discussion

In early 1961 it was reported that the activity of ampicillin against H. influenzae was better than that of penicillin, chloramphenicol and tetracycline. Since then it remained the drug of choice for treatment of serious infections due to H. influenzae until the emergence of ampicillin-resistant strains in 1974. All these isolates were found to have acquired ampicillin resistance because of their ability to produce beta-lactamases. By 1977 as many as 14–16% isolates in the USA were found to be ampicillin-resistant and their prevalence rose steadily to be between 6 and 34% in various parts of the world by 1986. The problem was compounded by development of resistance to alternative drugs like chloramphenicol, tetracycline and trimethoprim-sulphamethoxazole. During this study we found that 13% of our isolates were resistant to ampicillin compared with 19–20% in the USA, 10% in Europe, 14% in Korea, 17% in Thailand, 36% in Taiwan and 60% in Spain.

Resistance to other antimicrobial agents like chloramphenicol, erythromycin, tetracycline and trimethoprim-sulphamethoxazole have been reported in various parts of the world. Chloramphenicol and trimethoprim-sulphamethoxazole resistant strains are less common (0.5–0.7%) in the USA compared with those we found in Saudi Arabia and those reported from Europe and Asia. These findings prompted a search for effective yet safe drugs. Cefaclor is a second

Table 1

<table>
<thead>
<tr>
<th>Drug</th>
<th>Susceptible (%)</th>
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<tbody>
<tr>
<td>Cefaclor</td>
<td>98</td>
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<tr>
<td>Cephalothin</td>
<td>90</td>
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<tr>
<td>Ampicillin</td>
<td>87</td>
</tr>
<tr>
<td>Amoxycillin/clavulanic</td>
<td>95</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>47</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>93</td>
</tr>
<tr>
<td>Tetracyclin</td>
<td>77</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>95</td>
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</tbody>
</table>
| Trimethoprim-sulphamethoxazole | 65

*Although disc-diffusion susceptibility method was used, it correlates with MICs (mg/l) of equal to or less than 8.0 for cephalosporins, 2.0 for ampicillin, 4.0 for amoxycillin/clavulanic acid, 2.0 for erythromycin, 12.5 for chloramphenicol, 4.0 for tetracycline and gentamycin and 2/38 for trimethoprim-sulphamethoxazole.*
generation cephalosporin that has been found to inhibit *H. influenzae* both in vitro and in vivo. Since none of the studies in the past used isolates from Saudi Arabia, we investigated its in vitro activity against 383 recent clinical isolates. Of the total of nine antimicrobials used in this study, cefaclor was found to be most active, inhibiting 98% of the strains followed by augmentin, gentamicin and chloramphenicol. Erythromycin and trimethoprim-sulphamethoxazole were found to be least effective.

Pharmacokinetic studies have shown that cefaclor was well absorbed (90–95%) after oral administration in fasting subjects with the achievement of peak serum concentrations within 1 h. It is well distributed in most body fluids, achieving values exceeding MICs for *H. influenzae* and pneumococci in bronchial mucosa, sputum, saliva, middle ear fluid, biliary fluid and tears. Excellent in vitro activity, oral administration and pharmacokinetics make cefaclor a welcome addition in the treatment of acute otitis media, pharyngitis, purulent rhinitis, sinusitis and upper and lower respiratory tract infections caused by cefaclor susceptible strains of *H. influenzae*.

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References