Third generation cephalosporins and fluoroquinolones were compared (i) in an experimental animal model and (ii) by analysis of clinical data (Scavizzi et al., 1992; Gayraud et al., 1993); both these approaches led to the same conclusion: third generation cephalosporins—especially ceftriaxone—led to successes, but much less rapidly than fluoroquinolones, which appear to constitute the most suitable treatment in Y. enterocolitica infection.

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References  


Non-enzyme mediated β-lactam resistance in Haemophilus influenzae

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Sir,  
We read with interest the paper of James et al. (1996) regarding the high incidence of non-enzyme mediated amoxicillin resistance (13.6%) in Haemophilus influenzae isolated in Bath. The choice of amoxicillin as the test β-lactam differentiates this report from earlier studies which have almost exclusively concentrated on determining resistance to ampicillin (see review of Jorgensen, 1992).  

Early studies into comparative in-vitro activity of ampicillin and amoxicillin towards H. influenzae indicated an up to two-fold greater activity of ampicillin (Sutherland, Croydon & Rolinson, 1972; Rolinson, 1974). Despite the difference all strains studied at this time were highly sensitive, being inhibited by <1.0 mg/L of either agent. This lower in-vitro antibacterial activity for amoxicillin was compensated in vivo by increased serum levels compared to an equivalent dosage of ampicillin (Gordon, Regamey & Kirby, 1972). Therefore an unchallenged interchangeability for both agents occurred with the likelihood that ampicillin was tested in the laboratory and amoxicillin prescribed for the infection. Consequently, the British Society for Antimicrobial Chemotherapy (1991) recommended the same resistance breakpoint of 1.0 mg/L for both oral and iv formulations of amoxicillin and ampicillin.  

In-vitro studies of non-enzyme mediated resistance in H. influenzae to β-lactams quote ampicillin as the class representative and indicate varying but increasing rates of resistance throughout the world, ranging from 0.0 to 39% (Jorgensen, 1992; Ling, Lam & Cheng, 1993). In his analysis, Jorgensen (1992) make comment on discrepancies regarding the definition of ampicillin resistance between authors. Obviously such differences in the prevalence of resistance may be real or could be the product of methodological and or geographical variation.
Correspondence

Antibiotic | Number of isolates with MIC (mg/L):
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Ampicillin | <1.0 1.0 2 3 4 5 6 7 8 9 10
Amoxycillin | 33 13 9 2 13 10 19 7 3 3 1 1

*MICs measured by Etest method

Furthermore, analysis of such studies, in particular for non-β-lactamase mediated resistance, becomes even more confused when the β-lactam/β-lactamase inhibitor combination, amoxycillin plus clavulanic acid is included (Fuchs & Barry, 1994). That is, within the same study, test populations are being analysed against two β-lactams known to have differing in-vitro activities towards fully sensitive strains, but as yet unquantified differences towards intrinsically more resistant isolates. In the United States, before the availability of chromogenic cephalosporin substrates, a breakpoint of 1.0 mg/L was introduced to help identify β-lactamase positive bacteria from β-lactamase negative strains. Later, following the emergence of non-enzyme mediated resistance to ampicillin, a higher resistance breakpoint of 4.0 mg/L was introduced (NCCLS, 1990). Recently, Fuchs & Barry (1994) have recommended that because of differences in in-vitro activity and in-vivo serum concentrations, amoxycillin resistance should be re-defined as an MIC > 2.0 mg/L.

At this institution lower respiratory tract infections can be treated empirically with amoxycillin, with or without clavulanic acid, therefore isolates of *H. influenzae* are tested for susceptibility with low strength amoxycillin (2.0 μg disc) and amoxycillin/clavulanate (2.0/1.0 μg disc). However, it was our perception that we were seeing higher frequencies of non-β-lactamase mediated resistance towards amoxycillin than was recorded in the literature, albeit to ampicillin. Therefore we undertook a prospective study to determine the in-vitro difference in susceptibility to ampicillin and amoxycillin of *H. influenzae* which showed reduced inhibition zones to either amoxycillin (<15 mm to a 2.0 μg disc) and or cefuroxime (<19 mm to a 5.0 μg disc).

During a 12 month study period (January–December 1994), from a total of 541 isolates examined, 57 strains of β-lactamase negative *H. influenzae* with reduced inhibition zones to the two test β-lactams were identified. A further 73 (13.8%) isolates were shown to produce β-lactamase by nitrocefin hydrolysis (Unipath Ltd). Repeat controlled comparative disc sensitivity tests (using *H. influenzae* NCTC 45660 as the control) and Etest MICs (AB Biodisk Ltd.) were performed on 5% chocolate horse blood sensitivity agar (Unipath Ltd.) supplemented with Isovitalex solution (Becton Dickinson Ltd.) in 5% CO₂ in air. β-lactams tested (μg/disc) were, ampicillin (2 and 10) and amoxycillin (2 and 10). A summary of the results are shown in the Table. At the BSAC breakpoint (MIC > 1.0 mg/L) resistance rates for the 57 bacteria examined were, ampicillin 2.0% (n = 11) and amoxycillin 6.3% (n = 34). Only two strains had MICs > 2.0 mg/L for ampicillin compared to 15 for amoxycillin. On occasion inhibition zones for amoxycillin with both disc and Etest contained surviving colonies, suggesting a reduced bactericidal capacity compared to ampicillin. Scattergrams of MIC versus zones of inhibition were constructed and false sensitive and resistance rates were calculated. High strength (10 μg) discs of both β-lactams failed to clearly discriminate between sensitive and resistant strains. With a cut-off inhibition zone of 15 mm diameter no false resistance was detected with a 2.0 μg disc of ampicillin and the false sensitive rate was 3.5% (2/57, MICs of 1.25 mg/L). For amoxycillin, a cut off zone of 15 mm gave a false sensitive rate of 7.0% (4/57, MICs 1.25 to 1.5 mg/L) and no false resistance. Isolates having MICs of >2.0 mg/L to amoxycillin were characterised by inhibition zones of ≤13 mm to an amoxycillin 2.0 μg disc.

Concern regarding the MIC breakpoint chosen to define the prevalence of non-enzyme mediated resistance to β-lactams in *H. influenzae* has been expressed (Jorgensen, 1992). Our study shows that the β-lactam tested also contributes to the problem. At the BSAC breakpoint (1.0 mg/L), amoxycillin resistance (6.3%) was three-fold greater than for ampicillin (2.0%). If we adopt the breakpoint proposed by Fuchs & Barry (1994) of 2.0 mg/L for amoxycillin, to reflect its higher serum levels than ampicillin, our resistance rate would fall to 2.8% (15/541). In the paper of James et al. (1996) non-enzyme mediated...
Identification of mec-related oxacillin resistance in staphylococci by the Etest

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Sir,

Petersson, Miörner & Kamme (1996) reported in this Journal that the reliability of the Etest for the detection of mecA-related resistance in staphylococci has not been studied extensively. They concluded that separation of uninduced mecA-positive and negative strains was achieved by the Etest. Our experience shows that variable Etest results compared with MICs in MRSA can occur.

Two strains of MRSA isolated in this region were studied, of which the MRSA III-152 was a newly detected phage type in The Netherlands. This strain was found in a nursing home patient who had undergone eye surgery in a German hospital approximately one year previously. Despite the absence of special hygienic precautions the strain did not spread during a later hospitalization of the patient in the surgical department of the St. Jozef Hospital. In the De Wever Hospital, the MRSA phage type III-29 was the cause of a prolonged epidemic (Wagenvoort et al., 1993; 1996) and had also originally been imported in a patient transferred from a hospital in Germany. Moreover, in The Netherlands phage type III-29 is the most prominent strain imported from other European countries (Frenay et al., 1994).

Media employed were Mueller-Hinton agar (Oxoid CM 337) with 2% NaCl and PDM antibiotic sensitivity agar medium (AB Biodisk) with 2% NaCl (Merck 6404). The culture plates were either 1 or 4 days old and were incubated up to 48 h at 30°C or 37°C with a bacterial inoculum of 10^6 cfu per mL. The plates were employed for the disc diffusion test, the Etest and the agar dilution technique. MIC values for the MRSA strains were determined with methicillin and oxacillin Etest strips, applied according to the manufacturer's instructions. Disc diffusion zones with methicillin and oxacillin 5 μg discs (Oxoid) were measured. Inhibition zones of <17mm were considered as resistance. The disc diffusion and agar dilution techniques were those routinely performed for *Staphylococcus aureus*. Phage type and mecA gene determinations were performed by the State Institute of Public Health and Environmental Hygiene.

As the 1 day and 4 day old prepared agar media performed equally well, and the results read after 24 or 48 h incubation did not differ greatly, only the results after 24 h incubation were read.

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**References**


