Phenomenon of resistance to Augmentin associated with sensitivity to ampicillin: occurrence and explanation

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SUMMARY Ampicillin was found to be some tenfold more active than amoxycillin against *Enterobacter cloacae*. This finding explains the observation that some *Ent cloacae* strains are sensitive to ampicillin in the disc test but resistant to Augmentin. Ampicillin was also found to be more active than amoxycillin against *Citrobacter freundii* and *Serratia marcescens*. In view of these findings, the practice of using ampicillin discs to predict sensitivity to amoxycillin should be reconsidered. The use of both ampicillin and amoxycillin discs is appropriate if errors are to be avoided.

Amoxycillin has been shown to be at least as active in vitro as ampicillin against many pathogenic bacteria¹ with notable exceptions, such as *Haemophilus influenzae*. Its bactericidal action in vitro and its effectiveness in vivo seems to be superior to that of ampicillin.^{2,3} Further, amoxycillin is absorbed by mouth almost twice as well as ampicillin.¹ Because of these properties, amoxycillin can be given in a smaller dose and less frequently than ampicillin, and it now enjoys wide clinical use.

In the laboratory, it is recommended both in the UK and USA that sensitivity testing to amoxycillin be carried out using ampicillin discs.^{4,5} This is an example of what Barry and Thornsberry⁴ call "class" sensitivity testing, similar to that done in the USA by using a disc of cephalothin to determine sensitivity to the early cephalosporins (such as cephaloridine and cefazolin). The practice of "class" testing, however, may give highly misleading results if incorrectly applied. We have recently made some observations which cast serious doubts on the validity of "class" testing using an ampicillin disc. This investigation started when we isolated several strains of *Enterobacter cloacae* which appeared sensitive to ampicillin but resistant to Augmentin (amoxycillin + clavulanate) by disc testing (Figure). We briefly drew attention to this phenomenon, in a letter to the *Lancet*,⁶ and in this report we describe our findings in more detail.

Material and methods

ANTIBIOTICS

Ampicillin trihydrate and amoxycillin trihydrate were Laboratory Reference Standards nos 29 and 12, respectively, from Beecham Research Laboratories. Sodium ampicillin was obtained from the hospital pharmacy. Sodium amoxycillin and potassium clavulanate were generously supplied by Beecham Pharmaceuticals, Worthing.

BACTERIAL STRAINS

Twenty-nine strains of *Enterobacter cloacae*, 21 *Citrobacter freundii* and 13 *Serratia marcescens* were investigated. Four of the *Ent cloacae* strains had been isolated from patients who had recently received Augmentin, while all the other organisms were from our laboratory collection, which consists of bacteria freshly isolated from clinical material. Identification was by the API 20E system.

DISC SENSITIVITY TESTING

Augmentin discs (containing 20 µg amoxycillin plus 10 µg clavulanic acid) were obtained from Oxoid. We made discs containing 20 µg ampicillin, 20 µg amoxycillin and 20 µg ampicillin plus 10 µg clavulanic acid. This was done by impregnating Whatman AA discs with the appropriate dilutions of antibiotic.

Plates of Iso-Sensitest agar were flooded with a 1/100 dilution of overnight culture of the test organism. Plates were dried and then one of each of the

Both plates show results of disc testing with an *Enterobacter cloacae* strain (top half of each plate). The bottom part of each plate was inoculated with a sensitive control strain of *Escherichia coli*. AP25 = ampicillin 25 µg; A25 = amoxycillin 25 µg; AUG = amoxycillin 20 µg + clavulanic acid 10 µg. The results shown in left hand plate are confusing if ampicillin is used as a "class" test (see text). This apparently paradoxical result is explained by the result shown in the right hand plate, namely that the strain is sensitive to ampicillin but resistant to amoxycillin and Augmentin.

discs described above was placed on the surface. Plates were incubated overnight at 37°C and zone diameters measured with a millimetre rule. When zone sizes were being compared, a difference of 5 mm or more was regarded as being significant.

MIC DETERMINATIONS

These were made by a plate doubling dilution method with Iso-Sensitest agar. Inoculum sizes of 10^6 and 10^3 bacteria were applied to plates by means of a multipoint inoculating device (Denley); plates were read after overnight incubation.

DETECTION OF DESTRUCTION OF AMOXYCILLIN

Organisms were grown in a nutrient broth (3 ml) overnight at 37°C; 3 ml of a solution of amoxycillin (6 mg/ml in sodium phosphate buffer pH 8) was added and the mixture incubated at 37°C for 6 h. The amount of amoxycillin remaining was then assayed by the hydroxylamine method.⁷ It was found that strains either destroyed all the amoxycillin, or none at all. Strains which destroyed amoxycillin under these conditions are called "penicillinase-producing" strains.

Results

As stated earlier, we first observed the apparently paradoxical phenomenon of an organism being ampicillin-sensitive but Augmentin-resistant when testing *Ent cloacae*. We therefore screened other bacterial species for further evidence of the

phenomenon. It was detected only in *C freundii* and *S marcescens*, in both of which species it was unusual.

Twenty-one *C freundii* strains were examined. They fell naturally into two populations:

(a) 14 strains were sensitive to ampicillin, amoxycillin and Augmentin by the disc test. Ampicillin was slightly more active (two to threefold) than amoxycillin against these strains (Table). None of the 14 strains was a penicillinase producer, and clavulanate did not enhance the activity of either ampicillin or amoxycillin. Ampicillin was significantly more active than Augmentin in the disc test against three strains. (b) the remaining seven strains were resistant to ampicillin and to amoxycillin, but sensitive to Augmentin, in the disc test. MICs for these strains were about 100-fold higher than for sensitive strains (Table); ampicillin was again more active than amoxycillin. All seven strains produced penicillinase.

All 13 *S marcescens* strains showed rather small zones around the ampicillin, amoxycillin and Augmentin discs. Results of MIC determinations indicated that, overall, ampicillin was slightly more active (at most, twofold) than amoxycillin (Table). None of the strains produced penicillinase, and clavulanate did not enhance the activity of either ampicillin or amoxycillin. In four strains ampicillin was significantly more active than Augmentin.

Twenty-nine strains of *Ent cloacae* fell into two groups.

(a) 24 strains did not produce penicillinase; all were

Inhibitory concentration of ampicillin and amoxycillin against Enterobacter cloacae, Citrobacter freundii and Serratia marcescens strains. Data show that ampicillin and amoxycillin may have greatly differing activities, in terms of I50 and I90, for both large and small inocula.

Bacterial species	β -Lactamase activity	No of strains	Inhibitory concentrations (μ g/ml)							
			10 ³ organisms				10 ⁶ organisms			
			I50		I90		I50		I90	
			AMP	AMOX	AMP	AMOX	AMP	AMOX	AMP	AMOX
<i>Ent cloacae</i>	— +	24 5	7 >1024	80 >1024	30 >1024	410 >1024	14.5 >1024	128 >1024	52 >1024	530 >1024
<i>C freundii</i>	— +	14 7	2.7 210	6.4 800	7.6 >1024	15 >1024	6.6 330	8 900	15 >1024	60 >1024
<i>S marcescens</i>	—	13	15	11.5	50	70	27	58	100	120

* I50 and I90 = inhibitory concentrations of 50% and 90%, respectively, of strains tested.

† Ability to destroy amoxycillin (see methods).

sensitive to ampicillin by the disc test, and for 16 the zone size around the amoxycillin disc was at least 5 mm smaller than that around the ampicillin disc. Clavulanate did not appear to enhance the activity of either ampicillin or amoxycillin. The MIC of ampicillin was about one-tenth that of amoxycillin (Table). Fifteen strains in this group appeared to be sensitive to ampicillin but resistant to Augmentin. It is this type of resistance pattern which first suggested to us that *Ent cloacae* may be more sensitive to ampicillin than to amoxycillin. These 15 strains were distributed amongst eight different API biotypes. The other five strains destroyed amoxycillin, and were highly resistant to both ampicillin and amoxycillin (Table). They were also resistant to Augmentin and four were resistant to the ampicillin/clavulanate disc.

Discussion

As the first four strains of *Ent cloacae* resistant to Augmentin but sensitive to ampicillin which we isolated came within a few weeks from patients whom we had treated with Augmentin, our first thought was that these may be atypical strains which had been selected by the use of Augmentin. However, subsequently—over a period of some 12 months—we have only isolated one other such strain, from a patient given cotrimoxazole. This finding, and our observations that this type of *Ent cloacae* was common among unselected strains collected in the clinical laboratory before Augmentin came into use and that many API types are involved, suggests that such selection does not in fact take place.

Ent cloacae was not very common in our hospital: during 1981, *Enterobacter* spp accounted for only 1.7% of species isolated in significant numbers from

urines, and only 1.3% of blood culture isolates. It is therefore much less common than in USA, where this genus is responsible for between 4 and 10% of bacteraemias.⁸ *C freundii* is in our experience rarer than *Enterobacter* spp, and again much less commonly found than in USA⁹. Finally, *S marcescens* is virtually unknown in this hospital, although it has been reported from other centres in Great Britain.

The phenomenon of sensitivity to ampicillin coupled with resistance to Augmentin, which at first appeared to be an anomalous finding, is totally explained by the much greater activity of ampicillin (tenfold) than of amoxycillin against *Ent cloacae* (see Figure). The phenomenon was only noticed because we, like almost all other laboratories in this country, used "class" testing for determining sensitivity to amoxycillin. We now strongly recommend that where amoxycillin is in clinical use an amoxycillin disc be used for sensitivity testing, rather than ampicillin. This would also be helpful in the case of *Haemophilus influenzae*, against which ampicillin is also more active than amoxycillin.¹⁰ In view of the existence of organisms such as *Enterobacter* spp which show a differential sensitivity, testing against both ampicillin and amoxycillin discs may be necessary.

We were surprised that so many strains of *Enterobacter* spp and *S marcescens* were sensitive to ampicillin. These genera have been widely regarded as highly resistant to ampicillin, from the earliest days following the introduction of ampicillin. Before the advent of a rapid numerical taxonomic system—such as the API 20E system used in the present study—it was difficult to identify *Enterobacter* spp accurately, and indeed these strains used not to be separated from other biochemically related organisms, but were classified under the broad head-

ing¹¹ "Klebsiella-Enterobacter-Serratia". However, in a major study by Toala *et al.*,¹² in which *Ent cloacae* and *Ent aerogenes* were fully identified, none of 199 strains of either species was inhibited by 100 µg/ml of ampicillin. It may be that the API 20E system identifies organisms as *Enterobacter* spp which would have been classified as other species by conventional means.

It is perhaps coincidence that the genera in which we were able to detect the phenomenon of ampicillin sensitivity but resistance to Augmentin and amoxycillin all produce a type 1 β-lactamase. This type of enzyme is a cephalosporinase, which hydrolyses ampicillin very slowly.¹³⁻¹⁷ However, we have not found any data on the rate of hydrolysis of amoxycillin by this enzyme. Crump and Cansdale¹⁸ have suggested that clavulanate may induce type 1 β-lactamase, so that amoxycillin is destroyed more rapidly if it is present with clavulanate than if present alone. This type of mechanism has been shown to explain resistance of *Ent cloacae* to cefamandole in the presence of cefoxitin. The latter compound is an inducer of *Enterobacter* β-lactamase but inactive against this genus. The induced enzyme destroys cefamandole and thus causes resistance.¹⁹ Although we have not formally investigated the possibility that β-lactamase induction may occur, it seems *unlikely* to be of major importance in view of our finding that zone sizes around Augmentin discs were only rarely significantly smaller than those around amoxycillin alone. This effect was found for only three strains of the 63 tested (two *S marcescens* and one *C freundii*), and in no case did the presence of clavulanate decrease the activity of ampicillin.

Various other mechanisms have been called upon to explain the resistance of *Ent cloacae* to β-lactam antibiotics,^{20,21} and it seems that much more work will be necessary before the exact resistance mechanism in this intriguing genus are fully understood.

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