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Chromosomally mediated intrinsic resistance to penicillin of penicillinase producing strains of *Neisseria gonorrhoeae* isolated in Sydney: guide to treatment with Augmentin

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SUMMARY Single dose Augmentin treatment fails to cure an appreciable proportion of patients infected with penicillinase producing *Neisseria gonorrhoeae* (PPNG) strains in parts of the world where high levels of chromosomally mediated intrinsic resistance are also present in gonococci. The levels of intrinsic resistance to penicillin of 31 PPNG strains isolated in Sydney were assessed by obtaining ß lactamase negative variants of these strains and measuring the minimum inhibitory concentration of penicillin by agar plate dilution techniques. The levels of intrinsic resistance found in these imported PPNG strains were higher than those recorded for local isolates of non-PPNG strains, which indicates that caution should be exercised in the use of single dose Augmentin treatment of infections with PPNG strains in Sydney.

For many years penicillin and its analogues formed the basis of regimens for treating gonorrhoea. Resistance to the penicillins emerged in *Neisseria gonorrhoeae* through various mechanisms that were controlled by genes located either on the bacterial chromosome or extrachromosomally on plasmids. Chromosomal mutations may induce changes in penicillin binding proteins and in the permeability of the bacterial outer membrane protein to antibiotics. The net effect of these changes is known as the intrinsic resistance of the organism and is manifested as a series of incremental increases in gonococcal resistance to the penicillins. Acquisition of plasmids that code for the production of β lactamase is a second and distinct form of gonococcal resistance to the penicillins. This represents a single step change to high level penicillin resistance and is superimposed upon any underlying intrinsic resistance of the organism.

Augmentin is a potentially useful oral agent for treating infections caused by penicillinase producing *N gonorrhoeae* (PPNG) strains as the drug combines an inhibitor of β lactamase, clavulanic acid, with amoxycillin.[1] The clavulanic acid component has only weak antibacterial activity, which means that the outcome of treatment of infections with PPNG strains also depends on the intrinsic resistance of the organisms to the penicillins once the effect of the β lactamase of the organism has been neutralised by the clavulanic acid. Trials in England have reported success with this combination in the treatment of gonorrhoea, including infections with PPNG strains.[2,3] Other studies, however, most notably in Singapore, have recorded unacceptably high levels of treatment failure for patients infected with PPNG strains and treated with Augmentin.[4,5] Either a second dose of the combination, after an interval, or else the addition of procaine penicillin to the regimen was required for successful treatment.

The discrepant results reported for the outcome of Augmentin treatment of PPNG infections may be due to the different levels of intrinsic resistance encountered in various parts of the world. In South East Asia intrinsic resistance to the penicillins is high, so the beneficial effect of adding penicillin to Augmentin appears to relate to the capacity of this extra antibiotic to overcome these higher levels of intrinsic resistance, assuming that the β lactamase mediated resistance of the PPNG strains is neutralised by the concurrent administration of clavulanic acid.

In this study we sought to assess the levels of intrin-

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sic resistance to penicillin of PPNG strains isolated in Sydney as a guide to the possible outcome of treatment with Augmentin. We obtained this information by directly testing the sensitivity of β lactamase negative variants of PPNG strains.

Materials and methods

Strains of *N gonorrhoeae* were isolated on modified New York City medium and identified using rapid carbohydrate utilisation tests as described previously.[6] β Lactamase production was detected by acidometric and chromogenic cephalosporin tests.

ASSESSMENT OF INTRINSIC RESISTANCE TO PENICILLIN OF NON-PPNG STRAINS

Quantitative sensitivity tests were performed as described previously[7] by agar plate dilution techniques using Isosensitest agar (Oxoid) with added 8% saponin lysed horse blood, with an inoculum of 10^4 organisms delivered by a Steer's multiple inoculation device. Minimum inhibitory concentrations (MICs) of penicillin were assessed over the range 0·004 to 2 mg/l for 94 consecutive isolates of non-PPNG strains. Sixteen of these strains were randomly selected, and MICs of clavulanic acid for these 16 strains were assessed over the range 0·016 to 8 mg/l.

INTRINSIC RESISTANCE OF PPNG STRAINS

A single colony of each of 31 PPNG strains, isolated during the same period as the non-PPNG strains, was used to inoculate both a chocolate agar slope and a chocolate agar plate. After these were incubated for 24 hours at 36°C in 5% carbon dioxide in air, the slope was overlaid with sterile liquid paraffin[8] and stored at 37°C, and the growth on the chocolate agar plate was harvested and stored in 30% glycerol broth at −70°C. At one month intervals, subcultures were made from the paraffin overlaid slopes on chocolate agar plates inoculated and streaked to obtain single colonies. Individual colonies were tested for their ability to produce β lactamase, and colonies that lacked β lactamase activity were subcultured and stored at −70°C as for the parent strain. Before and after sensitivity testing, the identity of the strains was confirmed and their capacity to produce β lactamase re-examined. In addition, all strains and their variants were serotyped with Phadebact (Pharmacia, Australia) monoclonal antibodies to WI and WII/III antigens by coagglutination techniques.

The sensitivity to penicillin of the β lactamase negative variants of the PPNG strains was measured as for non-PPNG isolates (direct method). The MIC of penicillin for the 31 PPNG strains was tested over an extended range to 128 mg/l and also in the presence of 0·5 mg/l of clavulanic acid (indirect method). This concentration of clavulanic acid was included after preliminary experiments had shown that the 16 non-PPNG strains tested had MICs of clavulanic acid of 1·0 mg/l or more.

In addition, the chromosomally mediated resistance of the 31 PPNG strains and of their β lactamase negative variants to spectinomycin, ceftriaxone, and tetracycline was tested as an additional control on the identity of each parent PPNG strain and its derivative. The MIC of spectinomycin was assessed over the range 10–40 mg/l, that of tetracycline between 0·03–4 mg/l, and for ceftriaxone concentrations between 0·0005 and 0·03 mg/l were used.

Results

Table 1 shows the intrinsic resistance to penicillin of the 31 β lactamase negative variants of the PPNG strains examined. The MIC of penicillin for most strains was in the range 0·06–0·25 mg/l. One strain was inhibited by 0·03 mg/l of penicillin and the remaining six strains had higher MICs of penicillin, the highest level detected being 1·0 mg/l.

Also shown in Table 1 is the intrinsic resistance of

Table 1 *Intrinsic resistance to penicillin G (mg/l) of 31 PPNG and 94 non-PPNG strains*

Minimum inhibitory concentrations (MICs) by direct method of:*			*MICs of PPNG strains by indirect† method*			
	non-PPNG strains	*PPNG variants*	*0·125*	*0·25*	*0·5*	*1·0*
0·008	6	0				
0·016	2	0				
0·03	6	1				
0·06	47	3	1		2	
0·125	27	15	8	5	1	1
0·25	4	6	1		3	2
0·5	2	3		2		1
1·0	0	3			1	2
Total	94	31				

*Direct method, MICs assessed for β lactamase negative variants of PPNG strains.
†Indirect method, MICs assessed for parent PPNG strains in presence of 0·5 mg/l clavulanic acid. Intrinsic resistance of one strain, whose MIC was 0·03 mg/l by direct method, could not be assessed by indirect technique.

94 non-PPNG strains isolated over the same period as the PPNG strains. These strains have a lower level of intrinsic resistance to penicillin, with 14 strains being fully sensitive (MICs of 0·03 mg/l or less) to the antibiotic and only two strains with an MIC greater than 0·25 mg/l ($p < 0·01$)

Table 1 also compares the direct and indirect methods of assessing intrinsic resistance. In 24 of 30 β lactamase negative variants the levels of intrinsic resistance found by the direct method were the same as, or within one doubling dilution of, the value obtained in the parent PPNG strain by the indirect method. Discrepant results occurred in six PPNG strains with levels of intrinsic resistance higher by more than one doubling dilution by the indirect method. MICs for these strains were 0·06–0·25 mg/l by the direct method, but were 0·5–1·0 mg/l by the indirect technique. The growth of the remaining PPNG strain, whose MIC was 0·03 mg/l by the direct method, was inhibited in the presence of 0·5 mg/l of clavulanic acid. Comparisons between the values obtained by both methods could not therefore be made for this strain.

Table 2 shows the activity of the three other antibiotics tested against the 31 pairs of organisms. All isolates were fully sensitive to spectinomycin and ceftriaxone, but the strains were less sensitive to tetracycline. When the MICs of these antibiotics were assessed for each PPNG strain and its β lactamase negative variant, the levels recorded were identical or within one doubling dilution for each pair of organisms.

Seven strains were of serogroup WI and 24 were serogrouped as WII/III. In all cases the serogroup of the parent strain and the variant derived from it were identical. The non-PPNG strains isolated over the same period had a similar distribution of serogroups.

Discussion

This study showed that PPNG strains isolated in Sydney, which had subsequently lost their capacity to produce β lactamase, had raised levels of intrinsic resistance to penicillin and that these levels were higher than those of local isolates of non-PPNG strains. Six of the 31 strains tested showed high and the remainder raised levels of intrinsic resistance to this antibiotic and would be regarded as being relatively resistant or less sensitive to penicillin. Although most non-PPNG strains isolated in Sydney are less sensitive to penicillin, isolates categorised as relatively resistant to penicillin were uncommon in this and previous studies.[7 9]

Table 2 *Susceptibility of 31 PPNG strains and their β lactamase negative variants to spectinomycin, ceftriaxone, and tetracycline*

	Minimum inhibitory concentrations (range in mg/l) of:	
Antibiotic	*PPNG strains*	*β lactamase negative variants*
Spectinomycin	10	10
Ceftriaxone	0·002–0·016	0·001–0·016
Tetracycline	0·5–4	0·5–4

In several trials of Augmentin treatment of gonorrhoea caused by PPNG strains in Singapore, it was found necessary to increase the amount of penicillin administered to obtain acceptable cure rates.[5] It seems that similar strategies would be required to treat PPNG infections with Augmentin in Sydney because most PPNG infections seen in Australia are acquired in South East Asia. This consideration is given added weight by the findings reported here because the intrinsic resistance of the PPNG strains examined was in the range 0·03–1·0 mg/l. The 3·0 g amoxycillin contained in Augmentin is insufficient to guarantee the eradication of strains with levels of intrinsic resistance at the upper end of this range.[10] This situation would arise once the β lactamase of the organisms was neutralised by the clavulanic acid component of the antibiotic compound.

It is difficult to measure the intrinsic resistance of PPNG to the penicillins, and such assessments only became relevant when therapeutic combinations of penicillins and β lactamase inhibitors were available. Attempts were made to assess the intrinsic resistance of PPNG strains by indirect means by combining subinhibitory concentrations of clavulanic acid with various penicillins. The results of two such studies, however, differed as they reported different MICs of clavulanic acid.[11 12] In this study we took advantage of the observation that during storage PPNG strains lose their ability to produce β lactamase, presumably through the loss of the plasmid coding for this function. In contrast, the intrinsic resistance, being chromosomally controlled, should remain unaltered. By testing the β lactamase negative variants obtained from subcultures of single colonies of PPNG strains we were able to assess directly the intrinsic resistance to penicillin of these strains. Other characteristics that were also under the control of chromosomal genes were simultaneously measured in both the parent strains and their variants, and these observations were not altered by the plasmid curing procedure.

An attempt was made to assess indirectly the intrinsic resistance of these same PPNG strains by assessing the MIC of penicillin in the presence of clavulanic acid, but problems encountered in earlier studies were also met here.[12] It is difficult to arrive at a suitable concentration of clavulanic acid for such an assessment. We used an amount that was less than the MIC value, but this failed to neutralise all the β lactamase

produced by some strains, yet an increased concentration of clavulanic acid used in preliminary studies was inhibitory for other isolates.

PPNG strains are becoming increasingly important in gonococcal disease in Australia, particularly in the larger cities. The ultimate source of PPNG strains isolated in Australia is South East Asia, although endemic cycles of these imported strains have been established, particularly in Sydney.[9] The sensitivity of the PPNG strains and their β lactamase negative variants to other antibiotics was assessed to ensure that the plasmid curing procedures used did not affect chromosomally mediated functions of the organisms, but increased levels of resistance to tetracycline were observed coincidentally. This finding is not unexpected as resistance to tetracycline is linked to increased intrinsic resistance to the penicillins.[13] All strains were fully sensitive to spectinomycin and ceftriaxone, and either antibiotic would be suitable treatment for PPNG infections in Sydney. Some caution should be exercised, however, in the use of single dose Augmentin to treat PPNG infections in Australia.

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