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## Letters to the Editor

### Use of Intralactam for detection of β-lactamase production by *Neisseria gonorrhoeae*

In your Journal of July 1979 (p 738), DB Wheldon and Mary PE Slack reported an evaluation of Intralactam for the detection of β-lactamase production by *Haemophilus influenzae*. At the end of their paper they commented that it might also be used to detect gonococcal β-lactamase.

We have used this acridometric method to detect β-lactamase production in primary isolates of *Neisseria gonorrhoeae* for the past year. Initially we prepared paper strips impregnated with penicillin and indicator, as described by Slack *et al.*, but then began using the commercially available Intralactam.

All confirmed isolates of *N. gonorrhoeae* have minimum inhibitory concentrations (MIC) determined to penicillin, spectinomycin, and tetracycline. At first, testing for β-lactamase production was limited to isolates having an MIC to penicillin of > 2.0 mg/l. (Exact quantification of the MIC value above 2 mg/l was not performed.) Of 2357 *N. gonorrhoeae* isolated in this laboratory from clinical specimens in 1978-79, 38 organisms (1-6%) had an MIC to penicillin of > 2.0 mg/l. These 38 organisms had a β-lactamase determination performed, the majority using Intralactam strips, but a small number using our own prepared strips with penicillin and neutral red incorporated, as described by Slack *et al.* Of these 38 organisms, 36 were identified as β-lactamase producers. However, as MIC results and identification of β-lactamase production were not usually available to the clinicians until four days after primary isolation, we decided to perform β-lactamase determination on all isolates using Intralactam strips at the same time as a presumptive diagnosis of *N. gonorrhoeae* was made.

We now provide clinicians with a presumptive diagnosis of *N. gonorrhoeae* if oxidase-positive, Gram-negative diplococci are present on primary isolation plates after 18-24 hours' incubation, and at the same time report the presence or absence of β-lactamase production. The primary isolation medium used is modified New York City Medium.

We have used Intralactam strips to...
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determine β-lactamase production in 267 presumptive gonococcal isolates. Seven organisms had MICs to penicillin of > 2.0 mg/l, the remainder having MICs to penicillin of 0.03-1.0 mg/l. The Intra-lactam test identified the seven organisms with MICs of > 2.0 mg/l as β-lactamase producers, and none of the other 260 isolates gave a positive result with the test.

Due to Australia's proximity to south-east Asia, where β-lactamase producing N. gonorrhoeae strains are frequently encountered, we occasionally see such organisms in patients returning from these countries. There have also been some β-lactamase producing strains causing infections contracted locally. The standard treatment at the Venereal Diseases Clinic in Sydney is amoxicillin, 3 g orally, preceded by probenecid. This is highly effective except in the case of β-lactamase producing strains. For this reason we feel that rapid detection of β-lactamase production is desirable so that antibiotic treatment can be modified accordingly. In our experience, Intra-lactam strips provide a rapid, simple method for detecting β-lactamase production in N. gonorrhoeae.

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References
3 A human strain of Campylobacter fetus subsp. intestinalis grown at 42°C

A recent report of an infection with Campylobacter fetus subsp. intestinalis in a patient with multiple myeloma leads us to mention several unusual features of the case. The strain was first isolated from a presumably traumatic joint. A blood culture was requested following that isolation. Tryptic Soy Broth and Thio-glycollate Medium (w/o Indicator) bottles (Pfizer Inc), inoculated with 5 ml blood each, remained clear (both contained 12% CO₂, but the organism grew in a one-day subculture to chocolate agar at 37°C in 8% CO₂. Most remarkable, however, was the fact that the C. fetus isolate grew at both 25 and 42°C. Since it proved to be resistant to nalidixic acid (no zone around the 30 μg disc) and grew in 1% glycerine broth the identification of C. fetus subsp. intestinalis was established. Such strains have previously been reported from ovine sources but not from man. Conventional wisdom has it that the subspecies intestinalis and fetus (so far never isolated from man) grow at 25°C but not at 42°C while the subspecies jejuni grows at 42°C but not at 25°C. Our finding prompts us to re-emphasise that growth at 25°C is a more reliable characteristic in the differential diagnosis of C. fetus than growth at 42°C. For the differentiation of clinical strains, we therefore recommend growth at 25°C and susceptibility to nalidixic acid rather than growth at both 25°C and 42°C.

We also want to emphasise the importance of 'blind' subcultures of blood culture bottles to suitable media, such as chocolate agar in a CO₂ atmosphere. It is rare to see turbidity due to C. fetus in blood culture media, presumably because of an inadequate growth atmosphere in commercial bottles.

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References

Is there a place for the GammaCoat (125I) Gentamicin Radioimmunoassay Kit in a routine clinical pathology laboratory?

Waterworth has pointed out that a delay in obtaining results detracts from the value of a gentamicin assay service. In an attempt to minimise this delay a variety of rapid methods have been devised. All of these have their protagonists, but none has yet achieved general acceptance. Included among these rapid methods is radioimmunoassay in a number of forms. Radioimmunoassay techniques are simple, sensitive, and specific. Thus, when a kit, the GammaCoat (125I) Gentamicin Radioimmunoassay Kit (Clinical Assays Cat. No. CA-540), recently became available in this country, we made a preliminary assessment of it.

Paired (pre- and post-dose) sera from patients receiving gentamicin therapy, either alone or in combination with other antibiotics, were assayed by our current overnight plate diffusion method and then, together with standards used to calibrate this method and controls of varying values made up in the laboratory, re-assayed blind using the GammaCoat (RIA) kit. The principle of the Gamma-Coat technique and the recommended procedure for use of the kit are detailed in the instruction booklet. We followed the recommendations except that, for reasons of economy in the trial, only one tube per standard, control, or sample was used.

Altogether 109 samples were analysed by both methods. These comprised 15 standards, 12 controls, and 41 pairs of sera. The correlation between the results obtained by RIA and plate diffusion are shown in Figures 1 and 2. The linear correlation coefficient (r) for the standard/control results was +0.963 and for the patients' sera was +0.961. A slightly better correlation was obtained using a quadratic model (parabola) in which y (value by RIA method) = -1.22 + 1.49x + 0.038x² (where x = value by plate diffusion method) for the standard/control results and y = -0.23 + 1.21x + 0.0257x² for the patients' sera. Discrepancies seemed to be most marked at higher levels. Mahon et al. commented on discrepancies between the results by the two methods in their study and suggested that it might be due to the effects of multiple antibiotic therapy. This did not seem to be so in our case, other antibiotics present being inactive against our plate diffusion assay indicator organism, and a similar correlation being found for both patients' samples and standard/control samples. The RIA method may have been reading low at the higher levels, but we cannot explain why at present. An assessment of each pair of sera was made retrospectively by one of us (CDR), and it was found the variations...
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doi: 10.1136/jcp.33.5.508

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