

A problem with the recognition of penicillin-resistant pneumococci

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SUMMARY A strain of *Streptococcus pneumoniae* requiring 0.25 mg/l penicillin to inhibit its growth was isolated from the antrum of a child who had no history of either foreign contacts or previous treatment with penicillin. The penicillin resistance was recognised by chance because the disc used contained substantially less than its stated content of 1.5 unit. When discs containing 2 units were used there was little reduction in the size of the zone of inhibition.

It is now generally accepted that it can no longer be assumed that *Streptococcus pneumoniae* is sensitive to penicillin and that sensitivity tests must therefore be done. Despite this there have been only three reports of the isolation of resistant strains in this country.¹⁻³ We report a fourth, together with evidence suggesting that some of these organisms may pass unrecognised.

Clinical history

An asthmatic 5-year-old English boy presented with a history of intermittent hoarseness and headaches over a period of four months. On examination the nasal septum was found to be thickened and the postnasal space was slightly narrowed. Both antra were opaque on sinus x-ray. The chest x-ray was normal. There was no clinical evidence of immunodeficiency. He was admitted to hospital for three days, during which bilateral antral washouts were done. A heavy pure growth of *Haemophilus influenzae* was isolated from the washings of the left antrum.

The patient was seen again after six weeks, during which time he had received no antibacterial drugs. He was readmitted to hospital for 48 hours, and a repeat left antral washout yielded a heavy growth of *H. influenzae* and *Strep. pneumoniae*. After discharge from hospital he was given a course of cotrimoxazole, and when seen some eight weeks later he was well. Nose and throat swabs were taken at this time from both the patient and his mother, but no pneumococci were isolated.

Sensitivity tests

The original sensitivity test was performed with a disc containing 1.5 unit penicillin, and the organism gave only a very small zone of inhibition (about 3 mm from the edge of the disc). The test was repeated using a disc from a different batch containing 1 unit, and this produced a large zone not obviously smaller than that of the control staphylococcus. It was thus obvious, and was subsequently confirmed, that the original disc had deteriorated and contained considerably less than the stated 1.5 units.

The pneumococcus was then tested by the Stokes⁴ method on Oxoid Isosensitest agar containing 4% lysed horse blood, using the two discs containing 1 and '1.5' unit, together with a third containing 2 units. After overnight incubation there was again only a small zone of 2 mm round the '1.5' unit disc (control zone 9 mm). In contrast, with the discs containing 1 and 2 units the zones produced by the pneumococcus were respectively only 4 mm and 3 mm smaller than those given by the control. Thus, although this strain should be classed as only moderately sensitive when tested with the 1 unit disc, it would justifiably be classed as sensitive with the higher content disc (Fig. 1a). However, if the plate was examined by transmitted light there was a broad band of haemolysis and lysed colonies inside the two larger zones (Fig. 1b). Nevertheless, it is customary to measure zones from visible colonies. The experiment was repeated, and the zones were measured after both 7 and 24 hours' incubation. After 7 hours the zones around all three discs were

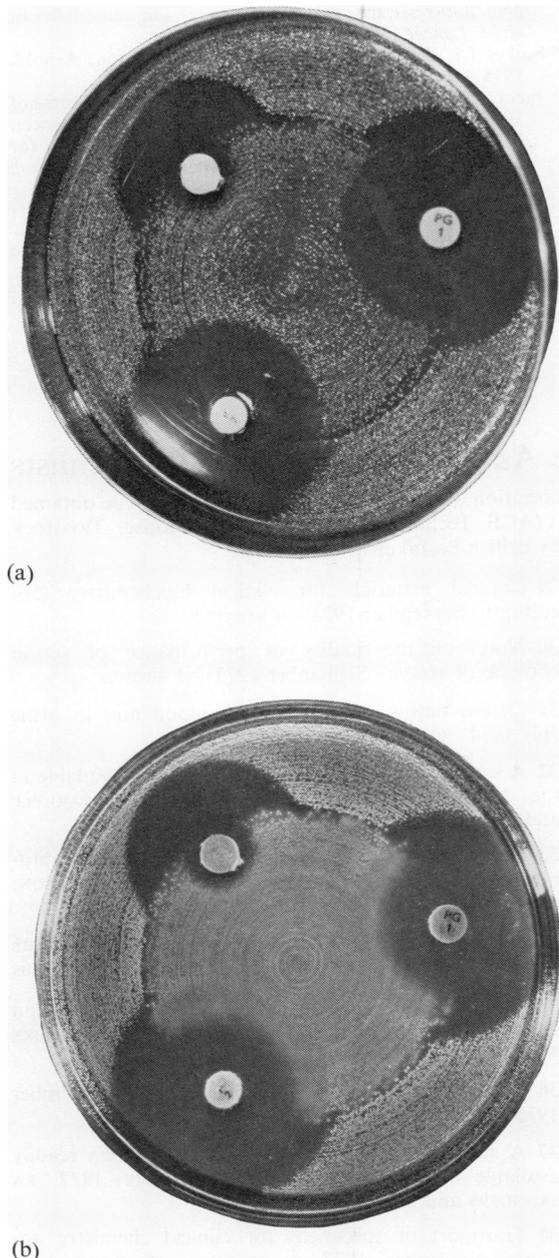


Fig. 1 Stokes test, on Oxoid Isosensitest agar + 5% lysed horse blood, comparing the sensitivity of the strain of *Strep. pneumoniae* (centre area) to that of the Oxford *Staph. aureus* (outer area). Discs contain: PG1 = 1 unit penicillin; P2 = 2 units penicillin; unlabelled disc = '1.5' unit penicillin. (a) Photographed by reflected light. The apparent distortion of the zones is caused by the angle from which the picture was taken. (b) Photographed by transmitted light. Note the absence of colonies in the band of lysis within the larger zones.

clear with well-defined edges and measured 3, 7, and 8 mm respectively. When examined the next day, that around the '1.5' unit disc showed little change but those around the discs containing 1 and 2 units had increased to 11 and 13 mm respectively, leaving an inner zone of haemolysis and lysed colonies. The staphylococcus control showed a 2 mm increase with both these discs.

The minimum inhibitory concentration (MIC) of penicillin for this strain of *Strep. pneumoniae* was determined by the plate dilution method using Oxoid Isosensitest agar with 5% lysed horse blood added and was found to be 0.25 mg/l, compared to a control strain of 0.015 mg/l. It was resistant to tetracycline by the disc test but sensitive to erythromycin and chloramphenicol.

Discussion

Penicillin-resistant pneumococci are apparently still very rare in this country, but the isolation of the present strain from a child with no history of previous penicillin treatment or foreign contacts infers that these strains may be present in the community. It is just possible that the patient acquired this strain by cross-infection during his brief stay in hospital, but, if so, the source has not been identified. No other comparable pneumococci have been isolated during routine microbiological studies up to the time of writing. It also seems possible that some strains may pass undetected. Several warnings have been given that these strains may not readily be recognised if high-content discs (10 units) are used, but it now seems that strains with moderate resistance may be difficult to recognise even when the customary low content disc is used. Certainly it seems possible that the present strain might have been missed but for the reduced disc content.

In a recent very extensive study of this subject in South Africa, Jacobs *et al.*⁵ obtained the best differentiation between sensitive, moderately resistant (MIC 0.1-1.0 mg/l), and resistant (MIC > 1.0 mg/l) strains with discs containing 0.15 µg (0.25 units), which may well be about the level of our '1.5' unit disc. It should also be remembered that *Strep. pneumoniae* will normally give a zone slightly larger than the Oxford staphylococcus, and any reduction in zone size must therefore be looked at critically. Nevertheless discs containing 2 units penicillin are clearly unsatisfactory for detecting resistance in *Strep. pneumoniae*.

We are grateful to Mr R Pracy, under whose care the patient was admitted, and to the MLSOs of the Bacteriology Laboratory of the Institute of Laryngology.

The photographs were taken by Mr P Luton, electron microscopist at University College Hospital.

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