and infectious disease excesses noted in the technicians was similar to the findings of a large scale survey of medical laboratory workers.³

The distinction between hazard and risk from occupational ill health is now the basis of the recently introduced Control of Substances Hazardous to Health Regulations (1988),⁸ which includes a requirement to assess the risks associated with exposure to microbiological as well as chemical agents in the workplace. Epidemiological investigations based on occupational sickness absence records such as this may assist occupational health staff in making such assessments.

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Multipoint microbiological assay for detecting β -lactamase

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Abstract

A multipoint microbiological assay for determining β -lactamase production by clinical isolates of bacteria was evaluated. With strains of *Haemophilus influenzae*, *Neisseria gonorrhoeae*, and *Branhamella catarrhalis* there was excellent correlation between results obtained using this method and those obtained using the chromogenic cephalosporin reference method. The multipoint method is an inexpensive yet reliable adjunct to conventional susceptibility testing methods.

The susceptibility testing of micro-organisms such as *Branhamella catarrhalis*, *Haemophilus influenzae*, and *Neisseria gonorrhoeae* using the agar-dilution method has been described.¹ One drawback of this method is that traditional indicators of β -lactamase activity—reduced zone size and increased colony size at zone edges around antibiotic discs—are absent. We believe *B. catarrhalis* poses a particular problem (unpublished observations). Although around 50% of strains are resistant to ampicillin as indicated by β -lactamase production,² many appear susceptible using the accepted ampicillin "break-point" of 1 mg/l.³ With *B. catarrhalis*, therefore, it is imperative to determine whether β -lactamase is produced if an agar dilution susceptibility testing method is to be used. Detection of β -lactamase production in *H. influenzae* and *N. gonorrhoeae* also has important clinical implications.

Diagnostic laboratories use four methods

for β -lactamase detection. Hydrolysis of the β -lactam bond yields a dibasic acid which can be detected by a change in pH—the acidometric method⁴ or by the ability to reduce iodine—the iodometric method.⁵ With the chromogenic cephalosporin,⁶ an electron shift in the cephalosporin molecule yields a coloured product. Loss of antibacterial activity may also be detected by microbiological assays which use susceptible bacteria to detect breakdown of β -lactam antibiotics by organisms which are applied by streaking, or on membranes or filter paper discs.⁷ The origins of such assays lie in clinical observations of "indirect pathogenicity".⁸ Of these methods, the microbiological assay has proved the easiest to adapt to multipoint methodology.

Methods

Diagnostic Sensitivity Test agar (DST:Oxoid) was supplemented with lysed horse blood (7%) and nicotinamide adenine dinucleotide (Boehringer Mannheim; 20 mg/l) to facilitate growth of fastidious organisms. An anti-swarming agent (1-4-Nitrophenyl-glycerol: 55 mg/l) and penicillin (0.02 mg/l) were added and the plate was surface seeded with *Staphylococcus aureus* (NCTC 6571: 10^5 cfu/ml). This plate was inoculated with the bacteria under test after the conventional antibiotic "break-point" set had been inoculated. Overnight incubation in 7% carbon dioxide showed a "satellite" of growth around organisms producing β -lactamase (figure).

To assess the reliability of the multipoint method we compared it with the chromogenic

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Control plate (left) and β -lactamase detection plate (right) showing "satellite" effect around β -lactamase producing colonies.

cephalosporin method for detection of β -lactamase.

Two drops of chromogenic cephalosporin solution (500 mg/l; Oxoid) were placed in each well of a 96 well microtitration tray. Three or four colonies of an overnight culture of the organism to be tested were suspended in 1 ml saline, and one drop of this was added to one well of the microtitration tray. The tray was incubated at room temperature and observed after one hour for positive results. The multipoint method was performed as described above using the same suspension. Positive and negative controls were used for both methods.

Results

We tested *B catarrhalis* (60 strains), *H influenzae* (60 strains), and *N gonorrhoeae* (30 strains) and found complete agreement between methods.

Discussion

The multipoint microbiological assay for detection of β -lactamase offers an inexpensive, easily controlled, yet reliable method for the detection of β -lactamase production, which can be added to present working protocols

with the minimum of disruption. Plates can be stored at +4 °C for two weeks without deterioration in the quality of the reaction.

We are currently undertaking further work to investigate if it is possible to obtain a substrate profile of the β -lactamase detected by incorporating different β -lactam antibiotics into the plates. We are also investigating the possibility of modifying the technique to detect other antibiotic inactivating enzymes.

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