References


New High-Content Disks for Determination of High-Level Aminoglycoside Resistance in Clinical Isolates of Enterococcus faecalis

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Disks impregnated with 500 and 1000 μg of streptomycin, 1000 μg of kanamycin and 250 and 500 μg of gentamicin were used for detection of high-level resistance to aminoglycosides in 120 clinical isolates of Enterococcus faecalis. Fifty-seven strains were highly resistant to streptomycin, 80 to kanamycin and 41 to gentamicin. Using disks containing 500 μg of streptomycin, 1000 μg of kanamycin and 500 μg of gentamicin

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strains resistant to high levels of these drugs (97.9 %, 100 % and 100 %, respectively) were accurately detected. Better discrimination between high-level and low-level resistance was achieved with a 500 μg streptomycin or gentamicin disk. Zone-size breakpoints are proposed for detection of high-level resistance by disk diffusion.

Most enterococci are not killed by clinically achievable concentrations of cell-wall active drugs administered alone. Bactericidal chemotherapy is recommended in cases of severe infections due to these organisms, bactericidal activity usually being obtained by combination of cell-wall active agents with aminoglycosides (1). However, the bactericidal synergy displayed by combinations is absent in the presence of high-level resistance to aminoglycosides (2). High-level resistance to streptomycin, kanamycin and gentamicin characterises the three major phenotypes of these organisms and can be routinely detected by several methods including agar dilution (3, 4), broth dilution (4–6) and agar disk diffusion (6–8).

Disk diffusion is a convenient and reliable method which is easy to perform in most laboratories. The purpose of this study was to evaluate disks containing various concentrations of streptomycin, kanamycin and gentamicin for detection of enterococci showing high-level resistance to these drugs.

Materials and Methods. One hundred and twenty non-replicate strains of Enterococcus faecalis were obtained in 1988 from clinical specimens at the Henri Mondor Hospital in Créteil, France. Enterococci were identified by colony morphology, Gram staining and biochemical characteristics (9). The MICs of streptomycin, kanamycin and gentamicin were determined by the agar dilution technique in Mueller-Hinton agar (Diagnostics Pasteur, France) with an inoculum of 10⁸ organisms per spot. Incubation was at 37 °C for 18 hours. Streptomycin was provided by Pfizer, France; kanamycin by Bristol Laboratories, UK and gentamicin by Schering, USA. Agar disk diffusion was performed in parallel to MIC determinations by flooding an inoculum of 5 x 10⁶–10⁷ cfu/ml onto Mueller-Hinton agar. Disks containing high concentrations of kanamycin (1000 μg), gentamicin (250 and 500 μg) and of streptomycin (500 and 1000 μg) were furnished by Diagnostics Pasteur. The perimeters of the aminoglycoside inhibition zones were clear-cut and the zone diameters could be easily measured.

The relationship between inhibition zone diameters and MICs was analysed with a computer programme written by Dr Le Van Thoi. For every aminoglycoside low and high MIC breakpoints were selected. Strains with aminoglycoside MICs equal to or above 2048 μg/ml were considered to have high-level resistance to this aminoglycoside. Strains with MICs of gentamicin, kanamycin and streptomycin equal to or below 64 μg/ml, 256 μg/ml and 128 μg/ml, respectively, were considered to have low-level resistance to these aminoglycosides. Strains with MICs between the two breakpoints were classified as indeterminate. The zone-size breakpoints giving a minimum of very major errors (false-susceptible) and major errors (false-resistant) relative to MICs were selected (10). A minor error comprised an indeterminate result by one of the techniques only.

Results and Discussion. The results obtained for streptomycin are presented in Figure 1. With the 500 μg disk a zone-size breakpoint of 12 mm allowed discrimination of 47 of the 48 strains highly resistant to streptomycin, a single strain being categorized as indeterminate. Fifty-eight strains were resistant to low levels of streptomycin (MICs ≤ 128 μg/ml) and exhibited zone diameters greater than 14 mm. The MICs of streptomycin for the 14 remaining strains were between 256 and 1024 μg/ml and corresponded to diameters ranging from 6 to 19 mm. Ten percent of minor errors and no major error were observed. A similar rate of errors was found when the 1000 μg streptomycin disk was used (Figure 1B). However, the 500 μg streptomycin disk was more convenient for detection of highly resistant enterococci since small zone diameters, nearly always ≤ 10 mm, were observed for these strains. All but one (with a zone diameter of 14 mm) of the 80 strains highly resistant to kanamycin grew at the fringe of the 1000 μg disk (Figure 2) and all resistant strains were detected if a zone-size breakpoint of 10 mm was used. In contrast, the zone diameters for all strains resistant to low levels of kanamycin were greater than 18 mm. Agreement between the MICs and results of the disk-diffusion technique using the proposed breakpoints was 100 % (Table 1). With gentamicin (Figure 3) both the 250 μg and 500 μg disks accurately detected 41 strains highly resistant to this drug. A single strain was indeterminate. Zone diameters in excess of 17 mm were measured for all strains resistant to low levels of gentamicin. The percentage of agreement was 100 % for this antibiotic. As expected, strains resistant to low levels of gentamicin displayed smaller zone diameters with the 250 μg