Evaluation of the Standardized Disk Diffusion and Agar Dilution Antibiotic Susceptibility Test Methods by Using Strains of Neisseria gonorrhoeae from Tucumán, Argentina

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At present, most Neisseria gonorrhoeae testing is done with β-lactamase and agar dilution tests using common therapeutic agents. Generally, in bacteriological diagnosis laboratories in Argentina, study of antibiotic susceptibility of N. gonorrhoeae is based on β-lactamase determination and agar dilution method using common therapeutic agents. The National Committee for Clinical Laboratory Standards (NCCLS) recently described a disk diffusion test that produces results similar to the reference agar dilution method for antibiotic susceptibility of N. gonorrhoeae. We obtained 57 gonococcal isolates from patients attending a clinic for sexually transmitted diseases in Tucumán, Argentina. Antibiotic susceptibility tests using agar dilution and disk diffusion techniques were compared. The established NCCLS interpretive criteria for both susceptibility methods appeared to be applicable to domestic gonococcal strains. The correlation between the minimum inhibitory concentration (MIC’s) and the zones of inhibition was studied for penicillin, ampicillin, cefotaxime, spectinomycin, cefoxatime, cephaloridine, cephalaxin, tetracycline, norfloxacin and kanamycin. Dispersion diagrams showed a high correlation between both methods, with a sensitivity of 89% and specificity of 91%.

Key words N. gonorrhoeae; disk diffusion; agar dilution; Argentina

The ability of bacteria to develop resistance to antimicrobial agents has become a significant problem in the treatment and control of communicable sexually transmitted diseases. This is particularly true for N. gonorrhoeae, which has evolved a number of different mechanisms to resist antimicrobial agents. These mechanisms are plasmid or chromosome encoded. The emergence of penicillinase-producing N. gonorrhoeae (PPNG) in 1976 has led to widespread high-level penicillin resistance. High-level tetracycline resistance (minimum inhibitory concentration, MIC ≥16 μg/ml) was reported for N. gonorrhoeae in 1985 by the Centers for Disease Control. Spectinomycin resistance has also been reported for many strains of N. gonorrhoeae.

There is an immediate need to simplify and standardize the in vitro antimicrobial susceptibility testing of N. gonorrhoeae, because of the frequency of isolation and the increased levels of antimicrobial resistance seen with these strains. Alternative methods, such as agar dilution antimicrobial testing, are generally laborious and not practical for routine use. The development of a standardized agar diffusion method for these tests is practical and allows fast and reproducible results for clinical microbiology laboratories if standards are observed. The purpose of this study was to evaluate the agar disk diffusion technique, comparing it with the agar dilution method proposed by the National Committee for Clinical Laboratory Standards (NCCLS), using strains of N. gonorrhoeae, isolated in Tucumán.

MATERIALS AND METHODS

Bacterial Strains Two gonococcal control strains (ATCC 49226: WHO A and WHO C) were selected to represent both susceptible (WHO A) and resistant strains (WHO C) for penicillin. In addition, 57 N. gonorrhoeae strains, isolated from samples from patients attending a clinic in Tucumán between 1990 and 1991, were studied.

Antimicrobial Agents Antimicrobial agents used for the agar dilution studies were penicillin, ampicillin, tetracycline, cefotaxime, norfloxacin, cefoxitin, spectinomycin, cephaloridine, cephalaxin and kanamycin, which were provided by the Microbiology Institute Carlos G. Malbran, as dry experimental substances with a known capacity. All drug solutions were prepared and stored according to the manufacturers’ instructions. The antimicrobial agent-containing disks included penicillin (10 U), tetracycline (30 μg), cefotaxime (30 μg) norfloxacin (10 μg), spectinomycin (100 μg), cefoxitin (30 μg), ampicillin (10 μg), cephaloridine (30 μg), cephalaxin (30 μg) and kanamycin (30 μg). All disks were stored in desiccated storage units at 4°C. The antimicrobial agents tested, their agar diffusion breakpoints and their MIC limits were determined according to the NCCLS.

Antimicrobial Susceptibility Testing The agar dilution method was performed according to the method established by the NCCLS. The disk diffusion method was performed with GC-agar using a 1% GC supplement. All agar plates (agar dilution and disk diffusion) were incubated at 35°C under a 5 to 8% CO₂ atmosphere, and results were read after 24h.

Data Analysis Linear regression analysis determined the relationship between the MIC and the disk zone diameter.

RESULTS

The isolated strains were highly susceptible to the antibiotics examined, except for tetracycline (12.54% of the strains showed resistance to this drug). Two strains out of 57 (3.50%) were resistant to penicillin, ampicillin and cephalosporin of the first generation (cephaloridine–cephalexin). The distribution of the MIC’s and the inhibition halos of the other antibiotics assayed were...
similar for both β-lactamase producing PPNG (plasmid encoded) and non-producing gonococci. The standard disk diffusion test allowed us to obtain reproducible results, when compared with the MIC method. Gonococci showed 1.76% more resistance to penicillin, cephalaxin and kanamycin with the disk diffusion test than with the MIC method. For tetracycline, the difference between both tests was 3.54% (the disk diffusion test showing more resistance again). Similar results were obtained in assays carried out by Stratton in 1984 and by Fekete in 1993.

With each type of antibiotic, a dispersion diagram was made to analyze the correlation between the susceptibility tests of the diffusion in agar and the MIC (Fig. 1). The index used for measuring the linear regression analysis grade is the Pearson coefficient (r). A strong correlation between both methods could be observed.

When estimating the sensitivity and the specificity of both methods taking the MIC as the standard value, and classifying the strains as resistant and non-resistant (sensitive), the following results were obtained: a sensitivity of 89% and a specificity of 91%. Similar results have been found by Jones et al., obtaining a correlation of 98% between both methods.
In conclusion, it can be said that the results of the disk diffusion test for this microorganism and for the tested antibiotics were perfectly reproducible and comparable with the dilution agar method (MIC) if standardized parameters are observed. Analysis with dispersion diagrams showed a high correlation between both methods, with a sensitivity of 89% and a specificity of 91%.

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