Comparison of Two Disc Diffusion Methods with Minimum Inhibitory Concentration for Antimicrobial Susceptibility Testing of Neisseria Gonorrhoeae Isolates

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Abstract

Background: A few studies are available comparing either minimum inhibitory concentration (MIC) values with the Clinical and Laboratory Standards Institute (CLSI) disc diffusion method or MIC with the Australian Gonococcal Surveillance Program (AGSP) method. Aim: This study was conducted with the aim to identify the most feasible and cost-effective method for antimicrobial susceptibility testing of Neisseria gonorrhoeae. Materials and Methods: Antimicrobial susceptibility testing of N. gonorrhoeae was performed using, in parallel, the E-test for MIC determination and disc diffusion by CLSI and AGSP techniques, and were compared. Susceptibility to penicillin, ciprofloxacin, tetracycline, ceftriaxone and spectinomycin and cefixime were determined by CSLI and AGSP method and Kappa statistics used to analyse the data with SPSS software. Results: All isolates were susceptible to ceftriaxone and spectinomycin by three methods. Ninety-nine (99%) strains were resistant to ciprofloxacin, while 1% showed intermediate susceptibility to ciprofloxacin by all methods. Statistically, there was a moderate level of agreement between the methods for penicillin. Conclusion: All three methods gave reproducible results. Although the media used in the disc diffusion by the AGSP method is easy and cheap to prepare, the CLSI method of disc diffusion testing is recommended for susceptibility testing of gonococcal isolates because of its feasibility and 100% accuracy, with MIC by E-test as the reference method.

Keywords: Antimicrobial susceptibility testing, MIC, Neisseria gonorrhoeae

Introduction

An important and essential measure for monitoring the effectiveness of recommended drugs for the treatment of gonorrhoea is surveillance of the in vitro antimicrobial susceptibilities of Neisseria gonorrhoeae.

In many countries, baseline data on the antimicrobial susceptibility of gonococcal isolates have not been collected. In the absence of such data, antibiotics to which gonococcal isolates are resistant may continue to be used. The establishment of such baseline data is especially important when syndromic approaches are used to diagnose and treat gonorrhoea. The antimicrobial susceptibility pattern of N. gonorrhoeae may change rapidly, especially in areas where ineffective treatment regimens are applied.

There are no universally accepted guidelines for testing the antimicrobial susceptibility of N. gonorrhoeae by a disc diffusion method, but different techniques are in practice, like the Clinical and Laboratory Standards Institute (CLSI) method, the Australian Gonococcal Surveillance Program (AGSP) method and the British Society for Antimicrobial Chemotherapy (BSAC) method. The recommended procedure for antimicrobial susceptibility testing of gonococci is a determination of the minimum inhibitory concentration (MIC by agar dilution or E-test). A few studies are available comparing either MIC values with the CLSI disc diffusion method or MIC values with the AGSP method. A comparison between all the three in vitro susceptibility methods has not been reported so far. In the present study, antimicrobial susceptibility testing of N. gonorrhoeae was performed by using, in parallel, the E-test for MIC...
Materials and Methods

Gonococcal strains

This study was conducted at the Department of Microbiology, Maulana Azad Medical College, New Delhi. A total of 100 consecutive N. gonorrhoeae strains were isolated from 89 (86.4%) of 103 men with urethritis, five (12.8%) of 39 women with endocervicitis and six (46.1%) of 13 sexual contacts of these patients.

The samples were inoculated directly onto the selective Modified Thayer Martin (MTM) medium and incubated at 35-36.5°C in moist air containing 5% CO₂ for 24-72 h. A candle jar was used for carbon dioxide production. The colonies suspected to be N. gonorrhoeae were presumptively identified by Gram stain and oxidase and superoxid tests.[10] Confirmation of identity after subculture on chocolate agar was based on the rapid carbohydrate utilization test (RCUT). Gonococcal isolates were stored at −70°C in tryptic soy broth (Difco Laboratories, Detroit, MI, USA) containing 20% glycerol.[10]

Antimicrobial susceptibility testing

All gonococcal isolates were examined for susceptibility to penicillin, ciprofloxacin, tetracycline, ceftriaxone and spectinomycin by the disc diffusion method of CLSI and the AGSP [Table 1].

MICs to all antibiotics were determined by the E-test. The E-test was performed as specified in the manufacturer’s (AB Biodisk) product package insert. World Health Organization strains A to E and N. gonorrhoeae ATCC 49226 were included as quality control. The interpretive criteria for all antibiotics except azithromycin were as recommended by the CLSI; criteria for interpretation of azithromycin were recommended by the Neisseria Reference Laboratory (NRL) at CDC.

β-lactamase production was assayed using nitrocefin discs (BBL Cefinase; Becton Dickinson, Harayana, India).

Statistical analysis

Data management and statistical analyses were performed using statistical software SPSS version 13.0. (Chicago Illinois, USA). Linear regression analysis was carried out to correlate the MICs by E-test and disc diffusion methods.

Kappa statistics and percent agreement were applied to determine the agreement between the methods.

Results

All isolates were found to be susceptible to ceftriaxone and spectinomycin by three methods. Ninety-nine (99%) strains were resistant to ciprofloxacin (QRNG), while one (1%) showed intermediate susceptibility to ciprofloxacin by all methods [Tables 2 and 3]. There was complete agreement between the CLSI, AGSP and MIC methods for ceftriaxone, spectinomycin and ciprofloxacin (kappa = 1).

For penicillin, one (1%), 67 (67%) and 32 (32%) isolates were interpreted as susceptible, intermediate susceptible and resistant, respectively, by the CLSI and MIC methods [Tables 2-4], while one (1%) 59 (59%) and 40 (40%) isolates were interpreted as susceptible, less sensitive and resistant, respectively, by the AGSP method. Eight isolates were less susceptible to penicillin by CLSI and MIC whereas they were resistant by AGSP [Table 5]. Statistically, there was a moderate level of agreement between the two methods for penicillin (kappa = 0.83).

Of the penicillin-resistant strains, 17 were found to be penicillinase-producing N. gonorrhoeae (PPNG) isolates. Multi-drug resistance (resistance to three or more antibiotics) was observed in 10 gonococcal isolates.

Discussion

We observed that only 1% of the isolates were sensitive to ciprofloxacin and penicillin, while 46% were sensitive to tetracycline. All isolates were sensitive to ceftriaxone and spectinomycin, which can therefore be used as the first-line drug for syndromic management of urethritis.

Our findings show complete agreement between the CLSI, AGSP and MIC methods for ceftriaxone, spectinomycin and ciprofloxacin. All the isolates (100%) were susceptible to ceftriaxone and spectinomycin, and 99% were resistant to ciprofloxacin by the three methods. However, for penicillin, using MIC by E-test as the gold standard, more isolates were labeled as being resistant by AGSP. Because interpretative criteria for azithromycin are not available by CLSI and AGSP, comparisons could not be done for azithromycin.

In India, to the best of our knowledge, our study is the first to compare two disc diffusion methods with MIC. Otherwise, few published reports have compared the E-test with the agar
Table 2: Antimicrobial susceptibility of *Neisseria gonorrhoeae* isolates by the CLSI and AGSP methods (n=100)

| Antibiotic disc | AGSP no. (%) | CLSI no. (%) |
|-----------------|--------------|--------------|
|                 | S            | LS           | R       | S            | I           | R       |
| Penicillin      | 1 (1)        | 59 (59)      | 40 (40) | 1 (1)        | 67 (67)     | 32 (32) |
| Tetracycline**  | NON-TRNG 80 (80) | TRNG 20 (20) | 48 (46) | 23 (23) | 31 (31) |
| Ciprofloxacin*  | 0            | 1 (1)        | 99 (99) | 0            | 1 (1)       | 99 (99) |
| Ceftriaxone     | 100 (100)    | 0            | 0       | 100 (100)    | 0            | 0       |
| Spectinomycin   | 100 (100)    | 0            | 0       | 100 (100)    | 0            | 0       |

S: Susceptible, I: Intermediate susceptible, R: Resistant, *Quinolone testing is performed with a combination of both nalidixic acid (30 µg) and ciprofloxacin (1 µg) discs. The category of susceptible, less sensitive or resistant for ciprofloxacin is determined by considering the annuller radius measurements obtained with both antibiotic discs. Ciprofloxacin (R=6, LS=6, S=6); nalidixic acid (R=0, LS=0, S=6). **Used to screen for high-level tetracycline resistance only. CLSI: Clinical and laboratory standards institute, AGSP: Australian gonococcal surveillance program.

Table 3: Antimicrobial susceptibility of *Neisseria gonorrhoeae* isolates by the E-test for MIC determination (n=100)

| Antibiotics       | Range (µg/mL) | MIC<sub>50</sub> (µg/mL) | MIC<sub>90</sub> (µg/mL) | S | I | R |
|-------------------|---------------|--------------------------|--------------------------|---|---|---|
| Penicillin        | 0.023-6       | 0.5                      | 3                        | 1 | 67 | 32***|
| Tetracycline      | 0.094-6       | 0.38                     | 24                       | 46 | 23 | 31****|
| Ciprofloxacin     | 0.38-32       | 2                        | 12                       | 0  | 1  | 99   |
| Ceftriaxone       | 0.002-0.023   | 0.006                    | 0.012                    | 100| 0  | 0    |
| Spectinomycin     | 1-8           | 6                        | 8                        | 100| 0  | 0    |
| Azithromycin      | 0.016-0.25    | 0.064                    | 0.19                     | 100| 0  | 0    |

S: Susceptible, I: Intermediate susceptible, R: Resistant, MIC:<sub>50</sub>, MIC:<sub>90</sub>: The MICs at which 50% of the gonococcal isolates tested were inhibited. **MIC:<sub>50</sub>: The MICs at which 90% of the gonococcal isolates tested were inhibited. ***Penicillínase producing N. gonorrhoea (PPNG): 17 (17%). ****High-level plasmid-mediated tetracycline resistance N. gonorrhoea (TRNG): 20 (20%). MIC: Minimum inhibitory concentration.

Table 4: Antimicrobial resistance of 100 isolates of *Neisseria gonorrhoeae* by the CLSI, AGSP and E-test methods

| Antibiotics         | AGSP No. (%) | CLSI No. (%) | E-test |
|---------------------|--------------|--------------|--------|
| Penicillin          | 40 (40)      | 32 (32)      | 32 (32) |
| Tetracycline*       | 20 (20)      | 31 (31)      | 31 (31) |
| Ciprofloxacin       | 99 (99)      | 99 (99)      | 99 (99) |
| Ceftriaxone         | 0 (0)        | 0 (0)        | 0 (0)  |
| Spectinomycin       | 0 (0)        | 0 (0)        | 0 (0)  |

*TRNG only, AGSP: Australian gonococcal surveillance programs, CLSI: Clinical and laboratory standards institute.

Table 5: Comparison of antimicrobial susceptibility of *Neisseria gonorrhoeae* isolates by the AGSP and CLSI, and E-test methods

| Antibiotics         | Category | E-test | Total | Kappa value |
|---------------------|----------|--------|-------|-------------|
| AGSP                |          |        |       |             |
| Penicillin          | S        | -      | -     | 1           | 0.83        |
| Tetracycline        | Not TRNG | 46     | 23    | 11          | 80          |

CLSI

| Antibiotics         | Category | E-test | Total | Kappa value |
|---------------------|----------|--------|-------|-------------|
| Penicillin          | S        | -      | -     | 1           | 1           |
| Tetracycline        | S        | 46     | -     | -           | 46          |

100% agreement in results for ciprofloxacin, ceftriaxone, spectinomycin (Kappa=1). AGSP: Australian gonococcal surveillance programs, CLSI: Clinical and laboratory standards institute.

The continual spread and ongoing emergence of resistance among *N. gonorrhoeae* isolates require that an accurate and simple method be performed to determine antimicrobial susceptibilities. We found that both disc diffusion methods showed a high degree of agreement with MIC values. All the three methods gave reproducible results. However, media used in disc diffusion by the AGSP method is easy and less expensive to prepare when compared with CLSI and E-test.

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[Table 6]. In addition, we can apply six discs per plate in the AGSP method; therefore, making the method overall more cost-effective. Although AGSP method is more economical, its feasibility depends on the availability of antimicrobial discs of low potency, and it requires more accuracy in measuring the inhibition zones. All the three methods can be easily performed in any routine diagnostic laboratory, but we recommend the CLSI method of disc diffusion for susceptibility testing of gonococcal isolates, because, firstly, it has 100% agreement with the E-test as the reference method for all the antibiotics, including penicillin and tetracycline; secondly, high potency
discs are easily available; and thirdly, it is technically less demanding in measuring the inhibition zones. Further studies on monitoring antimicrobial resistance of *N. gonorrhoeae* against all antibiotics, including azithromycin, and newer antimicrobial agents used in the treatment of gonorrhea need to be conducted and the best method of antimicrobial susceptibility testing for gonococcus needs to be identified.

Hence, the CSLI method of disc diffusion is the recommended method due to its higher feasibility and accuracy than the AGSP method.

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