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CORRELATION BETWEEN IN VITRO SUSCEPTIBILITY AND TREATMENT OUTCOME WITH AZITHROMYCIN IN GONORRHOEA: A PROSPECTIVE STUDY

P Khaki, *P Bhalla, A Sharma, V Kumar

Abstract

Purpose: This prospective study was carried out to determine the antimicrobial susceptibility of Neisseria gonorrhoeae isolates by disc diffusion method and minimum inhibitory concentration (MIC) by E-test with special reference to azithromycin. Also, the correlation between in vitro susceptibility and treatment outcome with single 2 g oral dose azithromycin was assessed. Methods: The study included 75 gonococcal isolates from males with urethritis, females with endocervicitis and their sexual contacts. All isolates were subjected to susceptibility testing for penicillin, ciprofloxacin, tetracycline, ceftriaxone, spectinomycin, cefixime and azithromycin. Males with gonococcal urethritis were randomised to receive a single dose of either azithromycin or ceftriaxone. Forty-two men with urethritis received 2 g single oral dose azithromycin, while all other patients were given 250 mg parental ceftriaxone. All patients were called for follow-up to assess clinical and microbiological cure rates. Results: While all the isolates were susceptible to ceftriaxone, spectinomycin, cefixime and azithromycin; 74 (98.7%), 24 (32%) and 23 (30.7%) strains were resistant to ciprofloxacin, penicillin and tetracycline respectively, by both disc diffusion method and E-test. The MIC range, \( \text{MIC}_{50} \) and \( \text{MIC}_{90} \) of N. gonorrhoeae strains, to azithromycin were 0.016-0.25, 0.064 and 0.19 \( \mu \)g/mL, respectively. Follow-up attendance of the patients was 52.4 with 100% clinical and microbiological cure rates. Conclusions: Results of our study indicate that 2 g single oral dose azithromycin is safe and effective in the treatment of uncomplicated gonorrhoea.

Key words: Antimicrobial susceptibility testing, azithromycin, gonorrhoea, Neisseria gonorrhoeae

The rapidly emerging antimicrobial resistance of Neisseria gonorrhoeae isolates to the currently recommended antibiotics, especially in areas where inefficient standard treatment regimens are applied, is a setback for effective treatment and control of gonococcal disease.1-3 Sentinel surveillance of the in vitro antimicrobial susceptibility of clinical isolates of N. gonorrhoeae has a crucial role in preventing spread of resistant strains and monitoring effective antimicrobial therapy for gonorrhoea.4,5

Although a successful outcome of antimicrobial therapy is conditioned by a number of factors, a good correlation between the level of in vitro susceptibility and the microbiological cure is essential for the prediction of treatment outcome. Strategies for the control of gonorrhoea have relied on the use of highly effective and, often, single-dose therapy administered at the time of diagnosis.6 Due to the high prevalence of fluoroquinolone resistance in certain parts of India,7,8 first line treatment with oral ciprofloxacin has been largely replaced by treatment with parenteral ceftriaxone. Another third-generation cephalosporin cefixime can be administered as an alternative oral therapy with efficacy being equivalent to that of ceftriaxone;9,10 however, the association of higher cefixime minimum inhibitory concentrations (MICs) with chromosomally mediated penicillin resistance may suggest a slowly rising trend of chromosomally mediated cephalosporin resistance.10

There are a few reports, which have shown an in vitro activity of azithromycin against N. gonorrhoeae and also demonstrated the efficacy of a 1 or 2-g single dose of this agent for treatment of gonorrhoea.11-15 Azithromycin has also been shown to have good activity against other sexually transmitted pathogens including Chlamydia trachomatis, Ureaplasma urealyticum and Haemophilus ducreyi.13,14,16 There is hardly any data about comparison of in vitro susceptibility of N. gonorrhoeae isolates to azithromycin and clinical efficacy of azithromycin in treatment of gonorrhoea in India.

Therefore, this study was carried out to compare the results of disc diffusion method with MIC values by E-test for azithromycin and we also conducted a prospective study to assess the correlation between in vitro susceptibility and treatment outcome with azithromycin in gonorrhoea.

Materials and Methods

Study population

The study population comprised 77 males with urethritis, 22 females with endocervicitis and 10 their sexual contacts attending the STD clinic of Lok Nayak Hospital, New Delhi, between April 2005 and March 2006. All patients
were included in the study after taking informed consent. A detailed history regarding demographic and clinical data was obtained from the patients. A full general physical and systemic examination was done before sample collection and treatment. Exclusion criteria were: antibiotic therapy with in the preceding four weeks, known hypersensitivity to macrolide antibiotic, serious cardiac, renal or hepatic disease, clinical evidence of disseminated gonococcal infection, other complications of gonorrhoea or untreated syphilis and any condition that might affect gastro-intestinal absorption of antibiotics (e.g., peptic ulcer disease, gastrectomy).

**Samples collection and processing**

Urethral specimens from males and endocervical specimens from females were collected for preparation of smears and inoculation of selective modified Thayer-Martin agar. The specimens were transported to the laboratory at room temperature inside a candle jar with a candle fit inside within 1 hour. The smears prepared from discharge were stained by Gram stain and examined under oil immersion (1000×). Presumptive diagnosis of gonorrhoea was made on the basis of presence of gram-negative intracellular diplococci within polymorphonuclear leukocytes.

**Treatment of patients**

Treatment was assigned to males with gonococcal urethritis using consecutive randomization with a predetermined 2:1 azithromycin-to-ceftriaxone ratio. Patients were randomised to receive either azithromycin (2 g oral dose) or ceftriaxone (250 mg parental). All patients were called for follow-up after 5-7 days to assess clinical response to treatment and to establish microbiological cure by collecting a repeat urethral endocervical sample for direct microscopy and gonococcal culture. Occurrence of any adverse drug reaction was also recorded.

**Isolation and identification of N. gonorrhoeae**

The inoculated plates were incubated at 35-36 °C in a humid atmosphere (70% humidity) containing 3-7% carbon dioxide for 24-72 h. A humid environment was created by placing a moistened cotton wool ball at the bottom of the candle jar. N. gonorrhoeae was identified by colony morphology, Gram stain, oxidase reaction, superoxol test and rapid carbohydrate utilization test. Gonococcal isolates were stored at −70 °C in tryptic soy broth (Difco) containing 20% glycerol.17

**Antimicrobial susceptibility testing**

All the isolates were examined for susceptibility to penicillin (10 IU), ciprofloxacin (5 µg), tetracycline (30 µg), ceftriaxone (30 µg), spectinomycin (100 µg), cefixime (5 µg) and azithromycin (15 µg) by the agar disc diffusion method.18 In addition, the MICs to all antibiotics except cefixime was determined by E-test. The E-test was performed as specified by the manufacturer (AB Biodisk). N. gonorrhoeae ATCC 49226 was included as quality control. The interpretative criteria for all antibiotics except azithromycin were as recommended by the Clinical and Laboratory Standards Institute (CLSI).18 Criteria for interpretation of azithromycin was recommended by the Neisseria Reference Laboratory (NRL) at CDC.19 β-Lactamase production was assayed using nitrocefin discs (BBL Cefinase; Becton Dickinson).17

**Statistical analysis**

Data management and statistical analyses were done using statistical software SPSS version 13.0. Chi-square test and Fisher’s exact test were used to compare the responses to therapy. Linear regression analysis was carried out to correlate the MICs by E-test and inhibition zone diameters by disc diffusion method.

**Results**

A total of 75 gonococcal strains were isolated from 67 (87%) out of 77 men with urethritis, 4 (18.2%) out of 22 women with endocervicitis and 4 (40%) out of 10 sexual contacts of these cases.

The antimicrobial susceptibilities of isolates are summarized in tables 1 and 2. All isolates were found to be susceptible to ceftriaxone, spectinomycin, cefixime and azithromycin. Seventy-four (98.7%), 24 (32%) and 23 (30.7%) strains were resistant to ciprofloxacin, penicillin and tetracycline, respectively. Thirteen (17.3%) strains were found to be PPNG and 15 (20%) were TRNG. Out of 24 penicillin-resistant strains, 13 (54.2%) were found to be PPNG and among the 23 tetracycline-resistant strains, 15 (65.2%) were found to be TRNG.

The MIC range, MIC₅₀ and MIC₉₀ of N. gonorrhoeae strains, to azithromycin were 0.016-0.25, 0.064 and 0.19 µg/mL, respectively (Table 2). Cut-off MIC values

| Antibiotic disc* | No. (%) |
|------------------|---------|
| Antibiotic disc* | S  | I  | R  |
| Penicillin       | 1 (1.3) | 50 (66.7) | 24 (32)** |
| Tetracycline     | 35 (46.6) | 17 (22.7) | 23 (30.7)*** |
| Ciprofloxacin    | 0 | 1 (1.3) | 74 (98.7) |
| Ceftriaxone      | 75 (100) | 0 | 0 |
| Spectinomycin    | 75 (100) | 0 | 0 |
| Cefixime         | 75 (100) | 0 | 0 |
| Azithromycin     | 75 (100) | 0 | 0 |

S - Susceptible, I - Intermediate susceptible, R - Resistant. *The interpretive criteria for disc diffusion were recommended by the Clinical and Laboratory Standards Institute (CLSI) and CDC Guideline. **Penicillinase producing N. gonorrhoeae (PPNG): 13 (17.3%). ***High-level plasmid mediated tetracycline resistance N. gonorrhoeae (TRNG): 15 (20%)
that were used to determine susceptibility were as per CSLI guidelines for all antimicrobial agents except azithromycin, for which CDC guidelines were used. The acceptable linear correlation between MIC values of azithromycin with the inhibition zone diameter around azithromycin disc was achieved with a regression coefficient value ($r$) of $-0.63$ (Figure).

Out of 75 patients, only 42 who were treated with single oral dose of 2 g azithromycin were included in the analysis of clinical and microbiological outcome. All were male patients between 18 and 54 years age group. Only 22 (52.4%) patients out of 42 cases came for follow-up after 5-7 days. All the patients (100%) were cured clinically, i.e., completely became asymptomatic and showed excellent bacteriological response with direct microscopy and culture for *N. gonorrhoeae* becoming negative. The most common treatment-related side-effect was mild diarrhoea (8%) followed by mild abdominal pain (1.6%).

**Discussion**

Increased resistance of *N. gonorrhoeae* isolates to oral fluoroquinolones has limited the options for effective treatment of gonorrhoea. Surveillance for antimicrobial resistance is crucial for monitoring the emergence and spread of antibiotic resistance in gonococcal isolates and to provide a rational basis for effective and affordable therapies for gonorrhoea.

Although the cost of azithromycin and the frequency of gastrointestinal intolerance are higher than those of alternative therapies and are likely to limit routine use of this regimen, azithromycin has several potential advantages for treatment. First, it is highly effective in the treatment of gonorrhoea with a single oral dose. Second, it provides appropriate treatment when the cause of the urethritis/cervicitis is uncertain and when immediate therapy is required before the results of bacteriological or serological tests are available. Finally, mixed gonorrhoea and chlamydial infection can be treated with a single agent.

Adequate *in vitro* results have been generated to recommend a breakpoint MIC ($\leq 1 \mu g/mL$) and a correlate zone diameter ($\geq 30$ mm). The results of some studies also have documented the clinical efficacy of a single oral dose of azithromycin (1 or 2 g) for treatment of gonorrhoea.

All gonococcal isolates were sensitive to azithromycin by the disc diffusion as well as *E*-test methods. Our study demonstrates the 100% clinical efficacy of single dose of 2 g azithromycin in the treatment of uncomplicated gonorrhoea in men and 100% correlation with the *in vitro* susceptibility results. Oral azithromycin may safely be recommended for treatment of uncomplicated gonorrhoea.

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**Table 2: Antimicrobial susceptibility of *N. gonorrhoeae* isolates by MIC (n = 75)**

| Antibiotics* | Range (µg/mL) | MIC 50 (µg/mL) | MIC 90 (µg/mL) | S | I | R |
|--------------|--------------|----------------|----------------|---|---|---|
| Penicillin   | 0.047-6      | 0.5            | 3              | 1 | 50| 24 |
| Tetracycline | 0.094-64     | 0.38           | 24             | 35| 17| 23 |
| Ciprofloxacin| 0.38-32      | 2              | 12             | 0 | 1 | 74 |
| Ceftriaxone  | 0.002-0.032  | 0.006          | 0.012          | 75| 0 | 0  |
| Spectinomycin| 1-12         | 6              | 12             | 75| 0 | 0  |
| Azithromycin | 0.016-0.25   | 0.064          | 0.19           | 75| 0 | 0  |

S - Susceptible, I - Intermediate susceptible, R - Resistant. *The interpretive criteria for MIC were recommended by the Clinical and Laboratory Standards Institute (CLSI) and CDC Guideline.

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**Figure:** Scattergram showing MIC values of azithromycin as compared to inhibition zone diameter around 15 µg azithromycin disc
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