International Journal of Antimicrobial Agents 35 (2010) 457–460

Contents lists available at ScienceDirect

International Journal of Antimicrobial Agents

journal homepage: http://www.elsevier.com/locate/ijantimicag

Antimicrobial susceptibility of *Neisseria gonorrhoeae* isolates determined by the agar dilution, disk diffusion and Etest methods: comparison of results using GC agar and chocolate agar

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ARTICLE INFO

Article history:
Received 23 November 2009
Accepted 5 January 2010

Keywords:
Antimicrobial susceptibility
Neisseria gonorrhoeae
Agar dilution
Disk diffusion
Etest
GC agar
Chocolate agar

ABSTRACT

Although the use of GC agar for determining *Neisseria gonorrhoeae* antimicrobial susceptibilities is suggested by Clinical and Laboratory Standard Institute (CLSI) guidelines, chocolate agar is still used in some regions owing to its low cost and availability. To determine the differences in susceptibilities determined using GC and chocolate agars, 163 non-duplicate *N. gonorrhoeae* isolates were tested. Minimum inhibitory concentrations (MICs) and percent susceptibilities determined using the GC agar dilution method, respectively, were as follows: ceftriaxone, 0.004–0.125 mg/L, 100%; cefixime, 0.002 mg/L to >32 mg/L, 98.2%; and ciprofloxacin, 0.002 mg/L to >32 mg/L, 3.1%. Comparison of ceftriaxone MICs determined by the Etest using GC agar and chocolate agar showed that use of GC agar tended to result in lower MICs than GC agar dilution, whilst use of chocolate agar tended to result in higher MICs (concordance, 55.8% and 82.8%, respectively). Disk inhibition zones obtained using GC agar and chocolate agar (and their correlation coefficients) were, respectively: ceftriaxone, 35–55 mm and 25–50 mm (0.46); ciprofloxacin, 6–55 mm and 6–43 mm (0.84); and penicillin, 6–47 mm and 6–50 mm (0.93). Use of chocolate agar with the disk diffusion method for ceftriaxone was associated with a 5.5% false resistance rate. In summary, compared with GC agar, susceptibility testing using chocolate agar tends to yield higher MICs with the Etest and smaller disk inhibition zones with disk diffusion methods. Clinical microbiology laboratories should strictly adhere to CLSI recommendations by using GC agar instead of chocolate agar when performing susceptibility testing for *N. gonorrhoeae*.

1. Introduction

Neisseria gonorrhoeae is a Gram-negative diplococcus and a major pathogen of sexually transmitted diseases [1]. It can cause urethritis, endocervicitis, proctitis and pelvic inflammatory disease with long-term sequelae, including infertility, ectopic pregnancy and adverse outcome of pregnancy [1]. In Taiwan, the incidence of *N. gonorrhoeae* is increasing and the number of confirmed cases of gonorrhoea reported to the Centers for Disease Control and Prevention increased from 361 in 2000 to 1437 in 2006 [2]. Resistance to antimicrobials is another challenge for the successful treatment of gonorrhoea. Penicillin resistance has been noted for decades worldwide [3,4] and quinolone resistance is emerging and is extremely high in Asian countries such as Taiwan [4–9]. Therefore, timely and accurate surveillance data on the distribution of resistance is helpful for clinicians to guide appropriate antimicrobial treatment.

Methods for testing antimicrobial susceptibility of *N. gonorrhoeae* still vary in different countries. Susceptibility testing using GC agar both in agar dilution and disk diffusion methods is suggested by Clinical and Laboratory Standards Institute (CLSI) guidelines in the USA [10,11] and also based on studies by Jones et al. [12,13]. However, a survey in Europe (European Surveillance of Sexually Transmitted Infections) showed that various kinds of methods and agar medium were used for *N. gonorrhoeae* susceptibility testing, including GC agar, diagnostic sensitivity test agar and chocolatised blood agar [14]. On the other hand, the World Health Organization has its own standard using chocolate agar plates comprising the Columbia agar base [15]. Some laboratories in Taiwan also use chocolate agar for the disk diffusion method owing to its low cost and availability. There are limited comparative data on

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doi:10.1016/j.ijantimicag.2010.01.007

the susceptibilities of *N. gonorrhoeae* determined using GC agar and chocolate agar [14,16]. This study compared the results of susceptibility testing of *N. gonorrhoeae* isolates using these two agars.

2. Materials and methods

2.1. Patients and hospital setting

Far Eastern Memorial Hospital is a 1000-bed hospital located in Taipei, northern Taiwan, providing daily outpatient service to ca. 5000 patients and emergency department services to over 300 patients. All non-duplicate *N. gonorrhoeae* isolates from clinical specimens during January 2006 to December 2007 were included in this study. Identification and analysis of antimicrobial susceptibility of these pathogens were routinely performed in a central laboratory.

2.2. Bacterial isolates

Isolates of *N. gonorrhoeae* were identified as oxidase-positive, Gram-negative, kidney-shaped diplococci with slightly concave adjacent surfaces in smears [1]. Chocolate agar and modified Thayer–Martin agar were used for isolation of bacteria [1]. Other confirmatory tests included the cystine trypticase agar (CTA) sugar test [1]. All isolates were stored in trypticase soy broth with 20% glycerol at −70 °C before further testing.

2.3. Agar dilution susceptibility testing using GC agar

Minimum inhibitory concentrations (MICs) were determined by the agar dilution method using GC agar (Difco GC medium base; BBL Microbiology Systems, Cockeysville, MD) and 1% defined growth supplement (IsoVitaleX^{TM}; BBL Microbiology Systems) according to CLSI guidelines [10]. Briefly, a Steers replicator was used to inoculate 0.5 McFarland standard of bacterial suspension onto GC medium containing a series of two-fold dilutions of tested antimicrobial agents. Following incubation (35 °C, 5% CO_2, 20–24 h), MICs were identified as the lowest concentration of antimicrobial agent that completely inhibited the growth of bacteria on the agar plate. *Neisseria gonorrhoeae* ATCC 49226 was used as the control strain. The following antimicrobial agents were tested: ciprofloxacin (Bayer Co., West Haven, CT); moxifloxacin (Bayer Co.); levofloxacin (Daiichi Pharmaceuticals, Tokyo, Japan); ceftriaxone (Roche, Basel, Switzerland); penicillin (Sigma Chemical Co., St Louis, MO); cefixime (Fujisawa Pharmaceuticals, Tokyo, Japan); tetracycline (Sigma Chemical Co.); and doxycycline and azithromycin (Pfizer, New York, NY).

2.4. Susceptibility testing by the Etest and disk diffusion method using both GC and chocolate agars

For further exploration of the results of susceptibility testing using chocolate agar (Chocolate II agar; BBL Microbiology Systems), ceftriaxone MICs of the isolates were further tested by Etest (0.016–256 mg/L) (AB BIODISK, Solna, Sweden) both on chocolate agar and GC agar. In addition, the disk diffusion method was performed with antimicrobials including ceftriaxone (30 μg), penicillin (10 U) and ciprofloxacin (5 μg) (BBL Microbiology Systems) using both agars and the inhibition zones were recorded [11].

2.5. Analysis

Determination of susceptibility category for the dilution and disk diffusion methods followed CLSI guidelines [10]. Agreement between ceftriaxone MICs determined by the Etest using both types of agar and those obtained by the agar dilution method using GC agar was determined; a difference between MICs of ±1 $\log_2$ dilution was defined as agreement. For comparison with the results of the agar dilution method, any ceftriaxone MIC obtained by the Etest that fell between two-fold dilutions was rounded up to the next two-fold dilution. Categories of susceptibility to penicillin, ceftriaxone and ciprofloxacin obtained with the disk diffusion method using two agars were compared with those obtained by the agar dilution method with GC agar.

Error rates of susceptibility categories for different agars were calculated. Acceptable error rates were <1.5% for very major errors (VME) (false susceptible; all resistant strains as denominator); <3% for major errors (MaE) (false resistant; all susceptible strains as denominator); and <10% for total errors (all inconsistencies were compared with the gold standard; total isolates as the denominator). Minor errors were defined as the total number of errors minus VME and MaE, divided by the total number of isolates [17].

3. Results

3.1. Bacterial isolates

A total of 163 non-duplicate gonococcal isolates was collected during the 2-year study period. Gonococci were isolated from 152 men (93.3%) and 11 women (6.7%). The age range of the patients with relevant isolates was 12–84 years. Isolates were predominantly from the urethra in men and from the cervix in women.

3.2. Antimicrobial susceptibility results

MICs for 50% and 90% of the organisms (MIC_{50} and MIC_{90}, respectively) and MIC ranges for the 163 isolates determined by GC agar dilution are shown in Table 1. Ceftriaxone and cefixime exhibited good activity against *N. gonorrhoeae*, with susceptibility rates of 100% and 98.2%, respectively. Susceptible rates to fluoroquinolones were all <4%. None of the clinical isolates were susceptible to penicillin or tetracycline.

3.3. Etest and disk diffusion susceptibility test results using chocolate agar

Ceftriaxone MICs determined by the Etest using GC and chocolate agar were compared with MICs determined by GC agar dilution (Table 2). The Etest MIC range with GC agar was 0.002–0.094 mg/L and with chocolate agar was 0.006–0.25 mg/L. Ceftriaxone MICs determined by the Etest using GC agar tended to be lower than by agar dilution, and MICs determined by the Etest with chocolate agar tended to be higher than by agar dilution (agreement, 55.8% and 82.8%, respectively).

Disk inhibition zones using GC agar and chocolate agar (and their correlation coefficients) were, respectively: ceftriaxone, 35–55 mm and 25–50 mm (0.46); ciprofloxacin, 6–55 mm and 6–43 mm (0.84); and penicillin, 6–47 mm and 6–50 mm (0.93). All isolates had ceftriaxone inhibition zones ≥30 mm on chocolate agar except for one isolate that had an inhibition zone of 25 mm. Compared with agar dilution, category agreement using chocolate agar for disk diffusion was 84.1% for penicillin, 95.1% for ciprofloxacin and 94.5% for ceftriaxone. The minor error, MaE and VME rates were 15.3% (25/163), 0% and 0.7% (1/137) for penicillin, 2.5% (4/163), 60.0% (3/5) and 0.7% (1/153) for ciprofloxacin, and 0%, 5.5% (9/163) and 0% for ceftriaxone. Using GC agar for disk diffusion, category agreement was 65.0% for penicillin, 93.3% for ciprofloxacin and 100% for ceftriaxone. The minor error, MaE and VME rates were 35.0% (57/163), 0% and 0% for penicillin, 4.3% (7/163), 60.0% (3/5) and 0.7% (1/153) for ciprofloxacin, and 0%, 0% and 0% for ceftriaxone (Table 3).