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E-Test method of antimicrobial susceptibility testing of *Neisseria gonorrhoeae* for routine diagnostic service

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Summary
The minimising inhibitory concentrations of four antimicrobial agents for 64 clinical isolates of *Neisseria gonorrhoeae* including 26 penicillinase producing strains (PPNG) as determined by E test, a recently developed method for sensitivity testing, were compared with those of agar dilution method using isosensitest (IST) agar. The medium was supplemented with either 5% lysed horse blood alone or with both lysed horse blood and 1% vitox defined supplement. The E test MICs compared closely with those obtained by agar dilution with essential agreements within ± 1 $\log_2$ dilution being over 90% with all test antibiotics on medium that did not contain vitox, and between 71 and 93% on medium containing vitox. The Pearson's correlation coefficients ranged from 0.84 to 0.96 on either medium formulation. Excellent categorical agreement was obtained for all isolates with ceftriaxone, ciprofloxacin and tetracycline, while the E test gave a minor categorical discrepancy for two isolates with penicillin. We conclude that the E test is as reliable as conventional agar dilution method for MIC testing of *N. gonorrhoeae* in a routine laboratory.

Keywords: *E-Test, susceptibility, gonococcus*

Résumé
La concentration minimal dintribution (MICs) de conissance de 4 agents antimicrobiens avait ete evalue chezx chuant 26 souche produite a la pencillinare (PPNG telque determine par le tests E. Ce teste avait ete compare a la methode de dilution de lagar ent utili sant le test d'agar Isosensitest (IST). Le milieu avait ate supplements srit avec du 5% du sang lysede cheval ou du sang de cheval lyse plus 1% de vitox. MICS du teste E avaient ete presque simi laire a ceux obtenuent a partir du teste de dilution d'agar avec une accordance essentrelle entre une dilution ± 1 de $\log_2$ pour plus de 90% de tours les antibiotiques testes sur les milieux sans vitrox. La correlation du coefficient de pearson etait comprise entre 0,84 et 0,96 Sur les 2 mi lieux. Les concondoances excellante et categorique avaient ete obtenus pour tous les isolats avec la ceftriaxone, ciprofloxacin et la tetracycline, alorsque le teste E avait donne une difference mineure categorique pour 2 isolats traite a la penicelline. Nous tirous done la conclusion solon laquelle le teste E est aussi sure que la teste de la methode donventionelle de la dilution sur agar pour da determination de la MIC de N gonorrhoeae dans les laboratoires de routine.

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Introduction

Emergence of resistance among clinical isolates of *Neisseria gonorrhoeae* to recommended antimicrobial agents [1] and the increasing clinical failure of various therapeutic regimens [2] have made it necessary to measure the antimicrobial susceptibility of isolates in clinical laboratories. Of the variety of techniques available for such susceptibility testing, the agar dilution method is the recommended procedure for clinical application [3]. However, the infrequent isolation of the organism makes this laborious and cumbersome procedure unattractive and impracticable on a routine basis with the result that it is very rarely employed in most laboratories. Consequently, it would be desirable to employ a simple and reproducible method that gives comparable results to agar dilution for routine determination of the MICs for *N. gonorrhoeae* in clinical laboratories.

E test (AB Biodisk, Solna, Sweden) is a recently developed method which combines the principles of disc diffusion and agar dilution methods of sensitivity testing by delivering an antimicrobial agent in a continuous gradient on one side of an impervious carrier strip from which it diffuses into the medium where a steady concentration is maintained along each level of the strip [4]. Studies comparing E-test with other methods of determining antimicrobial MICs for aerobic [5] and anaerobic [6] organisms have reported agreements usually greater than 90% between the methods. Similar studies on *N. gonorrhoeae* have been performed using GC agar base with comparable findings [7,8] In this study we evaluated the accuracy of the E test in determining the MICs of some commonly recommended antibiotics for 64 clinical isolates of *N. gonorrhoeae* using Iso-Sensitest agar containing different growth supplements.

Materials and methods

Sixty-four clinical isolates and two standard strains GC19424 and GC43069 of *Neisseria gonorrhoeae* with known penicillin susceptibility were used for this study. MIC determinations were performed in parallel on two occasions by agar dilution and E test methods on Iso-Sensitest (IST) agar (Oxoid, Unipath, England) supplemented with either 5% lysed horse blood alone or combined with 1% Vitox defined supplement (Oxoid, Unipath England). Standard powders of benzylpenicillin, tetracycline, ciprofloxacin and ceftriaxone were obtained from appropriate manufacturers, while E test strips of the same antibiotics were products of AB Biodisk. For each antibiotic, concentration ranges identical to those of the

Table 1: Distribution of isolates [No. (%)] by their E test MICs in relation to agar dilution $\log_2$

Antibiotic		>-2	-2	-1	Same	+1	+2	>+2	±1 $\log_2$	Corr. coefficient[a]
Penicillin	V+	4 (6.3)	6 (9.4)	29 (45.3)	13 (20)	4 (6.3)	7 (10.9)	1 (1.6)	46 (71.9)	0.84
	V-	1 (1.6)	3 (4.7)	32 (50)	21 (32.8)	6 (9.4)	1 (1.6)	-	59 (92.2)	0.92
Ceftriaxone	V+	-	4 (6.3)	8 (12.5)	43 (67.2)	7 (10.9)	1 (1.6)	1 (1.6)	58 (90.6)	0.93
	V-	-	1 (1.6)	4 (6.3)	50 (78.1)	5 (7.8)	4 (6.3)	-	59 (92.2)	0.96
Ciprofloxacin	V+	1 (1.6)	1 (1.6)	16 (25)	41 (64.1)	3 (4.7)	2 (3.1)	-	60(93.8)	0.91
	V-	-	2 (3.1)	8 (12.5)	46 (71.9)	7 (10.9)	1 (1.6)	-	61 (95.3)	0.94
Tetracycline	V+	1 (1.6)	6 (9.4)	15 (23.4)	21 (32.8)	15 (23.4)	4 (6.3)	2 (3.1)	51 (79.7)	0.89
	V-	2 (3.1)	1 (1.6)	14 (21.9)	38 (59.4)	8 (12.5)	1 (1.6)	-	60 (93.8)	0.94
Overall[b]		1.8%	4.7%	24.6%	53.3%	10.7%	4.1%	0.8%	88.7%	

[a] Pearson's correlation coefficient; V+ Medium containing vitox; V- Medium without vitox; [b] all antibiotics on both media

Table 2: Comparison of agreement and MIC ranges of Etest and agar dilution for PPNG with those of non- PPNG strains.

	MIC Range: mg/l agar / (Etest)		Essential agreement % with (without) vitox		Categorical agreement %with (without) vitox	
	PPNG	non-PPNG	PPNG	non-PPNG	PPNG	non-PPNG
Penicillin	4 – 256 (4 – 256)	≤0.016-4 (≤0.016-2)	70.8 (83.3)	77.5 (95)	100 (100)	95.8 (97.5)
Cefrtiaxone	≤0.016 – 0.12 (≤0.016 – 0.12)	≤0.016 - 0.12 (≤0.016 – 0.12)	91.7 (91.7)	87.5 (92.5)	100 (100)	100 (100)
Ciprofloxacin	≤0.008 – 0.25 (≤0.008 – 0.12)	≤0.008 – 0.25 (≤0.008 – 0.12)	91.7 (95.8)	95 (92.5)	100 (100)	100 (100)
Tetracycline	0.125 – 8 (0.125 – 4)	0.125 – 8 (0.125 – 4)	70.8 (87.5)	72.5 (97.5)	100 (100)	100 (100)

PPNG = Penicillnase producing Neisseria gonorrhoeae

corresponding E test strip were used for the agar dilution test. An inoculum of each test strain equivalent to 10^8 cfu/ml was prepared by emulsifying colonies from an overnight pure culture in 3 ml of digest broth to a density of 0.5 McFarland standard. The E test was performed by streaking this inoculum onto the two sets of IST agar and applying the E test strips 15 minutes after inoculation. For agar dilution, similarly supplemented IST media containing antibiotics in 2-fold increasing concentrations were inoculated with a 10-fold dilution in digest broth of the inoculum used for the E test, using a multipoint inoculator that delivered 10^4 cfu per spot. All plates were incubated at 35 °C in 5% CO_2 for 24 hours before reading of the MICs. As recommended by the manufacturers, any E test MIC lying between two values on the agar dilution scale is rounded up to the nearest higher value. All strains were tested for beta-lactamase production by the nitrocefin disc test.

Results

Twenty-four of the 64 strains tested were penicillinase-producing *N. gonorrhoeae* (PPNG), 9 were non-PPNG which were resistant to penicillin, while the remaining 31 were classified as penicillin susceptible having agar dilution MICs 0.06 mg/l or less. The standard strains were correctly tested by both agar dilution and E-test to have penicillin MIC of <0.015 mg/l. Comparisons of the E test MIC results with those of the agar dilution for all 64 isolates are shown in table 1. Essential agreement between the two methods, defined as E test MICs within 1 $\log_2$ dilution of agar dilution MIC for the antibiotics were: penicillin 71.9%, ceftriaxone 90.6%, ciprofloxacin 93.8% and tetracycline 79.7% on media containing vitox, while the corresponding agreement on medium without vitox were 92.2, 92.2, 95.3 and 93.8% and the overall essential agreement for all antibiotics on the media were 83.9 and 93.4%, respectively. The relationship obtained between the methods by Pearson's correlation coefficient were 0.84(0.92), 0.93(0.96), 0.91(0.94) and 0.89(0.94) for penicillin, ceftriaxone, ciprofloxacin and tetracycline respectively, on medium with (without) vitox. The antibiotic MIC ranges for the 24 PPNG strains and the essential agreements which range from 70.8 to 91.7% on medium containing vitox and 71.9 to 95.3% on medium without vitox are compared with those of non-PPNG strains in table 2. There is no significant difference between these essential agreement levels. The E test MICs were generally lower than those of the agar dilution on either medium. Categorical agreement between the methods was obtained with all test organisms for ceftriaxone, ciprofloxacin and tetracycline; while 2 non-PPNG strains which hadpenicillin MICs of 2 mg/l by agar dilution but 0.5 mg/l by E

test would have been classified as intermediate susceptible rather than resistant. All the isolates were sensitive to ceftriaxone and ciprofloxacin by both methods.

Discussion

Many studies have compared E test and agar dilution methods for determination of antimicrobial susceptibility of *N. gonorrhoeae* on various types of culture media, but to our knowledge no record is available of any similar study being performed on isosensitest agar which remains the routinely used medium for susceptibility testing in many laboratories. Adequate growth of all isolates was obtained on both media formulations used in this study to allow reliable MIC readings after 24 hours incubation. The overall essential agreements of 83.9% and 93.4% obtained in this study on medium with and without vitox respectively are similar to those reported by previous workers on various media types [7,9]. The essential agreement and correlation coefficient between the two methods are much higher when they are performed on medium supplemented with lysed horse blood alone than on medium that contains the vitox defined supplement as well. This poorer agreement and relationship is particularly pronounced with penicillin and tetracycline and have been previously observed by Yeung *et.al* when GC agar base is supplemented with both Kellogg's defined supplement and haemoglobin [9]. We have no explanation for the effect of vitox on the performance of the E test but note the report of Dillon *et al.* that different media formulations may have profound effects on various antibiotic MICs for *N. gonorrhoea* [10]. The E test in this study generally gave lower MICs than the agar dilution methods irrespective of medium used or antibiotic tested without any significant effect on the categorical agreements achieved. In spite of the large proportion of our isolates that were penicillinase producers and/or tetracycline resistant, the E test showed a categorical agreement of >96% with the dilution method for penicillin and 100% for other antibiotics. Although the E test correctly detected resistance in all our PPNG strains in contrast to the findings of Young *et al* [9] the frequent lower readings of the E test in the present study and the discrepant E test MIC of 0.5 mg/l rather than the 2mg/L by agar dilution for two non-PPNG strains supports earlier call for caution in the interpretation of penicillin E test MICs in the intermediate susceptibility zone [11]. The clinical essence of susceptibility testing of *N. gonorrhoeae* is to detect resistance to conventional anti-gonococcal agents in face of increasing therapeutic failure due to emergence of resistant strains. While penicillinase production easily detects penicillin resistance, those strains that are chromosomally resistant to penicillin can only be detected by MIC determinations. This is readily accomplished by the E test, which we find to be a reliable susceptibility test for the gonococcus on the frequently used routine 1st medium supplemented with 5% lysed horse blood. Addition of vitox defined supplement does not appear to improve the performance of the test.

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