

Brief reports

Failure of *Neisseria gonorrhoeae* to grow in the ATB NH susceptibility test system

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The ATB NH system designed for antibiotic susceptibility testing of *Neisseria gonorrhoeae*, *Neisseria meningitidis* and *Haemophilus influenzae* was evaluated using 94 clinical isolates of gonococci representing a wide variety of serovar/auxotype strains. Using the manufacturer's automated system 55% of the clinical isolates failed to grow, compared with a 33% failure rate for manual processing and visual reading. Growth failure was significantly higher with 1A isolates (73% automated and 69% manual) than with 1B isolates (49% automated and 25% manual). The higher failure rate of 1A isolates correlated with multiple auxotrophy. The inability of the ATB NH system to support the growth of common serovar/auxotypes makes the ATB NH system unsuitable for antibiotic susceptibility testing of *N. gonorrhoeae*.

Introduction

Gonorrhoea is once again increasing in prevalence in the UK,^{1,2} reversing the marked decline which occurred during the late 1980s and the first half of the 1990s. Although the majority of patients with gonorrhoea are treated on the basis of an established treatment protocol without access to the antibiotic susceptibility pattern of the individual isolate, nevertheless susceptibility testing is important to monitor the resistance pattern of isolates circulating within a community, and to formulate and review treatment policies.³ The nature and level of gonococcal antibiotic resistance as well as the spectrum of test methods and strategies have been reviewed recently.⁴

ATB NH (bioMérieux, Lyon, France) is a new commercial system designed to test isolates of *Neisseria gonorrhoeae*, *Neisseria meningitidis* and *Haemophilus influenzae* against a range of antibiotics impregnated in wells on a plastic strip. However, as there are no publications dealing with gonococci, the aim of this study was to evaluate the ATB NH system for susceptibility testing of *N. gonorrhoeae*.

Materials and methods

Bacterial strains

Ninety-four clinical isolates of *N. gonorrhoeae* (24 penicillinase producers) were used, comprising 26 serogroup 1A and 68 serogroup 1B isolates representing a wide variety of different serovars: 1A2 (6); 1A4 (3); 1A5 (2); 1A6 (5); 1A7 (1); 1A16 (4); 1A21 (3); 1A25 (2); 1B1 (10); 1B2 (7); 1B3 (7); 1B5 (2); 1B6 (8); 1B7 (5); 1B8 (4); 1B15 (3); 1B17 (7); 1B19 (2); 1B25 (2); 1B26 (1); 1B29 (2); 1B31 (1); non-typable (7).

ATB NH test system

Each test kit contains plastic strips which each have two rows of 16 wells. The first pair of wells do not contain any antibiotic and act as a growth control: the remaining 15 pairs of wells are impregnated with various antibiotics. The kit also contains ATB PBS suspension fluid and ATB S growth medium. All kits were stored at 2–8°C in the dark, as recommended, and all were within their expiry date at the time of use.

ATB NH testing

Tests were performed according to the manufacturer's instructions and used an overnight culture on modified New York City medium lacking antibiotics to prepare the inoculum in ATB PBS. After inoculation the strip was covered with a plastic lid and incubated at 35–37°C in an atmosphere of 5% CO₂. The test system was evaluated on two separate occasions. In the initial manual evaluation the inoculum suspension was prepared to a visual turbidity equal to a number 4 McFarland optical standard and a manual pipette was used to inoculate each well with 135 µL of suspension. Results were read visually after 24 h incubation and again after 48 h incubation (referred to as Visual 1 in Results).

In the second evaluation a bioMérieux densitometer and automatic pipette were used to standardize the test suspensions and inocula to the antibiotic wells. After incubation the tests were read visually (referred to as Visual 2 in Results) and by the ATB automated reader (referred to as Automated in Results). Gonococcal growth was indicated by visual turbidity or by a pre-set value on the automated reader. If there was no growth in the control wells, the results were not valid. The system classifies strains as susceptible or resistant depending on whether or not they grow in the wells containing antibiotic.

Conventional MIC testing

The same overnight culture plate that was used to make the number 4 McFarland optical standard in ATB PBS for the initial manual ATB NH testing (see above) was also used to make a number 1 McFarland suspension in saline for parallel MIC testing⁵ using a multi-point inoculator to inoculate a series of antibiotic-containing media and a control plate lacking antibiotic.

Statistical analysis

Differences were analysed by the χ^2 test using the Minitab PC package.

Results and discussion

On initial manual testing 33% (31/94) of the strains failed to grow in the ATB NH test system although all of the isolates grew on the conventional MIC control plate which was inoculated at the same time with a less dense suspension made from the same culture. The correlation between growth, ATB NH test protocol, and serovar/auxotype of the 26 1A isolates and the 68 1B isolates is given in Table I and Table II, respectively.

The overall performance of automated reading, which failed to detect growth in 55% (52/94) of isolates, was significantly poorer ($P<0.01$) than the 37% (35/94) failure with visual reading 2, and 33% (31/94) with visual reading 1. This poorer performance of the automated reading was only seen with serogroup 1B isolates (Table II), but not with serogroup 1A isolates. The failure rate of 49% (33/68) for the automated reading with 1B isolates is significantly higher ($P<0.01$) than the 19% (13/68) and 25% (17/68) failure rates for visual readings 1 and 2, respectively. In contrast, the failure rate of 73% (19/26) for 1A serovars by automated reading was not significantly different ($P<0.7$) from the 69% (18/26) failure rate by visual readings 1 and 2 (Table I). In each case, however, the overall failure rate with 1A isolates was significantly higher than with 1B isolates: 69% versus 19% for visual reading 1 ($P<0.001$); 69% versus 25% for visual reading 2 ($P<0.001$); and 73% versus 49% for automated reading ($P<0.05$).

Failure to grow was not uniformly distributed throughout the serovar/auxotype combinations. Auxotrophic strains failed to grow more often than prototrophic strains.

Table I. Correlation between growth and serovar/auxotype for 26 serogroup 1A clinical isolates

Serovar/auxotype	No. tested	Visual 1 growth		Visual 2 growth		Automated growth	
		Yes	No	Yes	No	Yes	No
1A2/NR	1	0	1	0	1	0	1
1A2/AHU	5	0	5	0	5	0	5
1A4/NR	3	3	0	3	0	2	1
1A5/AHU	1	0	1	0	1	0	1
1A5/NT	1	0	1	0	1	0	1
1A6/NR	3	3	0	3	0	3	0
1A6/A	2	1	1	2	0	2	0
1A7/NT	1	0	1	0	1	0	1
1A16/AHU	4	1	3	0	4	0	4
1A21/AHU	3	0	3	0	3	0	3
1A25/AHU	2	0	2	0	2	0	2
Total 1A	26	8	18	8	18	7	19

Auxotype: requirement for A—arginine, P—proline, H—hypoxanthine, U—uracil; NR, non-requiring; NT, non-typable.

Gonococcal growth and ATB NH susceptibility testing

Table II. Correlation between growth and serovar/auxotype for 68 serogroup 1B clinical isolates

Serovar/auxotype	No. tested	Visual 1 growth		Visual 2 growth		Automated growth	
		Yes	No	Yes	No	Yes	No
1B/NR	8	7	1	7	1	4	4
1B1/PAHU	1	0	1	0	1	0	1
1B1/PAU	1	0	1	1	0	0	1
1B2/NR	6	6	0	5	1	3	3
1B2/PAHU	1	1	0	0	1	0	1
1B3/NR	2	2	0	1	1	2	0
1B3/A	1	1	0	1	0	1	0
1B3/P	4	2	2	3	1	2	2
1B5/A	1	1	0	1	0	1	0
1B5/P	1	1	0	1	0	1	0
1B6/NR	3	3	0	3	0	3	0
1B6/P	5	3	2	5	0	4	1
1B7/NR	2	2	0	1	1	1	1
1B7/P	3	3	0	3	0	3	0
1B8/NR	1	1	0	1	0	0	1
1B8/A	2	1	1	0	2	0	2
1B8/P	1	1	0	0	1	0	1
1B15/PA	3	1	2	3	0	3	0
1B17/NR	7	7	0	6	1	0	7
1B19/P	2	2	0	2	0	1	1
1B25/P	2	2	0	0	2	0	2
1B26/PHU	1	0	1	0	1	0	1
1B29/PAU	2	1	1	0	2	0	2
1B31/NR	1	1	0	1	0	1	0
NT/NR	7	6	1	6	1	5	2
Total 1B	68	55	13	51	17	35	33

Auxotype: requirement for A—arginine, P—proline, H—hypoxanthine, U—uracil; NR, non-requiring; NT, non-typable.

This is illustrated by visual reading 1. With 1A isolates, the failure rate was 14% (1/7) for prototrophic strains (NR isolates) compared with 88% (15/17) for auxotrophic isolates ($P<0.001$). With 1B isolates, the failure rate was 5% (2/37) for the prototrophic strains and 35% (11/31) for the auxotrophic strains. The higher failure rate of 1A auxotrophs could be related to multiple auxotrophy: 58% (15/26) of the 1A isolates required more than one growth factor compared with 13% (9/68) of the 1B isolates ($P<0.001$). This is in keeping with the finding that commercial pre-poured plate media for isolating gonococci perform significantly less well ($P<0.001$), with serogroup 1A isolates supporting growth of 86% of isolates compared with 99% growth for serogroup 1B isolates.⁶

The panel of strains chosen in this evaluation is representative of strains which are prevalent in many geographical locations. Serovar 1A-2 has been shown to account for the majority of serovar 1A infections and also to account for as much as 97%⁷ and 98%⁸ of AHU strains. Auxotrophy is less common in 1B isolates and accounted for 36%⁷ and 37%⁹ in non-selected 1B populations.

The possibility that the ATB PBS was toxic for some gonococcal strains cannot be excluded on the basis of our experimental design. However, the highly significant correlation between growth failure and auxotrophy strongly supports the view that lack of growth was due to a deficiency in the ATB S growth medium. The inability to detect growth of common serovar/auxotypes makes the ATB NH system unsuitable for antibiotic susceptibility testing of *N. gonorrhoeae*. Growth detection was poorer with the proprietary automated system than by manual preparation and visual examination. Results were similar after incubation for 48 h. Because of the very high level of growth failure we did not compare susceptibility patterns determined by the ATB NH system and conventional MIC testing.

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