ORIGINAL ARTICLE

Quality assurance for antimicrobial susceptibility testing of *Neisseria gonorrhoeae* in Latin American and Caribbean countries, 2013–2015

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ABSTRACT

Objectives A *Neisseria gonorrhoeae* antimicrobial susceptibility quality control comparison programme was re-established in Latin America and the Caribbean to ensure antimicrobial susceptibility data produced from the region are comparable nationally and internationally.

Methods Three panels, consisting of *N. gonorrhoeae* isolates comprising reference strains and other characterised isolates were sent to 11 participating laboratories between 2013 and 2015. Antimicrobial susceptibilities for these isolates were determined using agar dilution, Etest or disc diffusion methods. Modal minimum inhibitory concentrations (MICs) for each panel isolate/antibiotic combination were calculated. The guidelines of the Clinical and Laboratory Standards Institute were used for interpretations of antimicrobial susceptibility. The agreement of MICs with the modal MICs was determined for each of the participating laboratories as well as for each of the antibiotics tested.

Results Five of 11 laboratories that participated in at least one panel had an overall average agreement between participants’ MIC results and modal MICs of >90%. For other laboratories, agreements ranged from 60.0% to 82.4%. The proportion of agreement between interpretations for all the antibiotics, except penicillin and tetracycline, was >90%. The percentages of agreement between MIC results and their modes for erythromycin, spectinomycin, cefixime and azithromycin were >90%. Tetracycline, ceftriaxone and ciprofloxacin agreement ranged from 84.5% to 89.1%, while penicillin had 78.8% agreement between MICs and modal MICs.

Conclusions The participating laboratories had acceptable results, similar to other international quality assurance programmes. It is important to ensure continuation of the International Gonococcal Antimicrobial Susceptibility Quality Control Comparison Programme to ensure that participants can identify and correct any problems in antimicrobial susceptibility testing for *N. gonorrhoeae* as they arise and continue to generate reproducible and reliable data.

INTRODUCTION

There are 78 million cases estimated of gonorrhoea reported annually worldwide.1 Over the past decades, *Neisseria gonorrhoeae* has developed resistance to all classes of the antimicrobials used for treatment including sulfonamides, penicillins, tetracyclines and fluoroquinolones. Isolates with reduced susceptibilities to the third generation cephalosporins and resistance to azithromycin have emerged in the last few years.2 The WHO endorsed Gonococcal Antimicrobial Susceptibility Programme (GASP) has been in place in Latin America and the Caribbean (LAC) since the 1990s.3 4 In 2012, the WHO published recommendations to control the global spread of antimicrobial resistance in *N. gonorrhoeae* and one of the key recommendations was to enhance surveillance in GASP and strengthen laboratory capacity.5 Quality-assurance systems are critical to ensure that the antimicrobial susceptibility data produced by different laboratories are accurate, standardised and comparable, nationally and internationally. Reference cultures with higher minimum inhibitory concentrations (MICs) to the third generation cephalosporins, azithromycin, ciprofloxacin and spectinomycin were provided by the WHO for laboratories to use in internal and external quality assurance programmes.6

A new gonococcal antimicrobial susceptibility quality control (QC) comparison programme, revitalising a QC programme begun in the 1990s, was initiated for the GASP-LAC countries.4 Participants included laboratories in Chile, Colombia, Cuba, Paraguay, Uruguay, Venezuela, Panama, Argentina and Peru, as well as the Focal Point for the GASP-LAC. The QC programme was coordinated and distributed by the Coordinating/Focal Point Centre for the GASP-LAC (Saskatchewan, Canada) and the National Microbiology Laboratory (NML), located in Manitoba, Canada. This report presents data for three panels from the International Gonococcal Antimicrobial Susceptibility Quality Control Comparison Program collected from 2013 to 2015.

METHODS

The first panel tested comprised eight WHO isolates (F, G, K, L, M, N, O and P) plus ATCC 49226 strains that are used for QC comparisons. The first panel was not blind. The other two panels included five *N. gonorrhoeae* isolates currently circulating in Canada in addition to control strains ATCC
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Table 1  Performance of participating laboratories in the International Gonococcal Antimicrobial Susceptibility Comparison Program between 2013 and 2015

<table>
<thead>
<tr>
<th>Laboratory code</th>
<th>Test method used</th>
<th>GC agar brand/supplement*</th>
<th>No. of panels tested</th>
<th>Antibiotics tested†</th>
<th>Per cent agreement between MICs of participant and modal MICs</th>
<th>Per cent concordance between interpretations of participant and modal interpretations</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Kirby-Bauer</td>
<td>Oxoid/Vitox</td>
<td>1</td>
<td>X X X X X X</td>
<td>72.2% (13/18)</td>
<td>81.5% (22/27)</td>
</tr>
<tr>
<td>2</td>
<td>E-test</td>
<td>Oxoid÷in house/Vitox</td>
<td>3</td>
<td>X X X X X X X X</td>
<td>82.0% (109/133)</td>
<td>92.0% (160/174)</td>
</tr>
<tr>
<td>3</td>
<td>E-test/agar dilution†</td>
<td>Difco/Isovitalex</td>
<td>3</td>
<td>X X X X X X X</td>
<td>97.0% (129/133)</td>
<td>99.5% (188/189)</td>
</tr>
<tr>
<td>4</td>
<td>E-test</td>
<td>Oxoid/Vitox</td>
<td>1</td>
<td>X X X X X X</td>
<td>94.1% (16/17)</td>
<td>100.0% (24/24)</td>
</tr>
<tr>
<td>5</td>
<td>E-test</td>
<td>Oxoid/Vitox</td>
<td>2</td>
<td>X X X X X X</td>
<td>70.7% (29/41)</td>
<td>83.6% (61/73)</td>
</tr>
<tr>
<td>6</td>
<td>Agar dilution</td>
<td>Oxoid÷Difco/Kellogg</td>
<td>3</td>
<td>X X X X X X X</td>
<td>91.7% (110/120)</td>
<td>94.0% (158/168)</td>
</tr>
<tr>
<td>7</td>
<td>E-test</td>
<td>Oxoid/Isovitalex</td>
<td>2</td>
<td>X X X X X</td>
<td>82.4% (14/17)</td>
<td>100.0% (24/24)</td>
</tr>
<tr>
<td>8</td>
<td>Agar dilution</td>
<td>Difco/Britalex</td>
<td>1</td>
<td>X X X X X X X</td>
<td>97.5% (79/81)</td>
<td>96.0% (96/100)</td>
</tr>
<tr>
<td>9</td>
<td>Agar dilution</td>
<td>Oxoid/Vitox</td>
<td>1</td>
<td>X X X X X X X</td>
<td>79.6% (43/54)</td>
<td>87.1% (54/62)</td>
</tr>
<tr>
<td>10e</td>
<td>Agar dilution</td>
<td>Difco/Isovitalex</td>
<td>1</td>
<td>X X X X X X X</td>
<td>60.0% (9/15)</td>
<td>64.7% (22/34)</td>
</tr>
<tr>
<td>12</td>
<td>Agar dilution</td>
<td>Difco/Kellogg</td>
<td>2</td>
<td>X X X X X X X</td>
<td>93.5% (86/92)</td>
<td>93.4% (128/137)</td>
</tr>
</tbody>
</table>

*Media and supplements purchased in respective countries.
†Etest Pen, penicillin; Spec, spectinomycin; Tet, tetracycline; Ery, erythromycin; Cx, ceftriaxone; Cip, ciprofloxacin; Ce, cefixime; Azi, azithromycin.
§Only one control and three test isolates were available for testing.
¶Only one control and four test isolates were viable for testing.

49226, WHO F, WHO G, WHO K and WHO P. Isolates were shipped on chocolate agar slants covered with sterile paraffin oil and/or Amies clear transport medium (Thermomisher, Burlington, Ontario, Canada). Each participating laboratory tested the isolates with the antimicrobials and methodology routinely employed in their laboratory (agar dilution, Etest or disc diffusion) (10). Six laboratories used the agar dilution method, four used Etest and only one reported disc susceptibility testing (table 1). One laboratory used both agar dilution and Etest. The medium used was GC Agar base with supplements as shown in table 1. Results were submitted to the NML and analysed using the Labware Laboratory Information Management System V.6.0 with IBM Cognos V.10.2. Disk diffusion zones were converted to approximate MICs, and Etest MIC results were rounded up to the closest twofold dilution value for comparison with agar dilution results. Modal MICs for each panel isolate/antibiotic combination were calculated from the modified data excluding the results presented.

Categorical interpretations for resistance (R) and decreased susceptibility (DS) were applied to the MIC results (including MICs with a ‘≤’ or ‘≥’) submitted by the participating laboratories as follows: penicillin (R≥2 mg/L), tetracycline (R≥2 mg/L), ciprofloxacin (R≥1 mg/L) and spectinomycin (R≥128 mg/L) using Clinical Laboratory Standards Institute guidelines (11); cefixime (DS ≥0.25 mg/L) and ceftriaxone (DS ≥0.125 mg/L); erythromycin (R≥2 mg/L) (12); azithromycin (R≥2 mg/L). (13) Modal categorical interpretations were determined for each panel isolate/antibiotic combination. The per cent concordance between categorical interpretations was determined for each participating laboratory as well as for each antibiotic.

RESULTS

Five of the 11 laboratories that participated in at least one panel of this quality programme from 2013 to 2015 achieved an overall average agreement between participants’ MIC results and modal MICs of >90%, which is considered the acceptable standard. The remaining laboratory’s agreements ranged from 60.0% to 82.4%, (14, 15) Concordance between the participating laboratories’ interpretations and the modal MIC interpretations was >90% for seven laboratories with the remaining

Table 2  MIC and categorical interpretation agreement among participating laboratories for each antibiotic

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Total no. of results</th>
<th>Total no. of comparisons</th>
<th>Modal MIC range (mg/L)</th>
<th>Modal MIC (±1 log.)</th>
<th>Categorical interpretation</th>
<th>Total number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillin</td>
<td>174</td>
<td>99</td>
<td>0.25–64</td>
<td>78.8% (78/99)</td>
<td>86.5% (141/163)</td>
<td>3/16</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>166</td>
<td>129</td>
<td>0.5–32</td>
<td>89.1% (115/129)</td>
<td>80.0% (124/155)</td>
<td>16/22</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>59</td>
<td>22</td>
<td>1–128</td>
<td>100.0% (22/22)</td>
<td>97.7% (43/44)</td>
<td>6/11</td>
</tr>
<tr>
<td>Spectinomycin</td>
<td>94</td>
<td>88</td>
<td>8–16</td>
<td>90.9% (80/88)</td>
<td>97.9% (92/94)</td>
<td>1/26</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>174</td>
<td>102</td>
<td>0.004–0.125</td>
<td>86.3% (88/102)</td>
<td>95.2% (157/165)</td>
<td>7/19</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>174</td>
<td>110</td>
<td>0.004–32</td>
<td>84.5% (93/110)</td>
<td>98.2% (167/170)</td>
<td>3/22</td>
</tr>
<tr>
<td>Cefixime</td>
<td>86</td>
<td>41</td>
<td>0.008–0.25</td>
<td>92.7% (38/41)</td>
<td>96.4% (81/84)</td>
<td>2/15</td>
</tr>
<tr>
<td>Azithromycin</td>
<td>138</td>
<td>126</td>
<td>0.125–16</td>
<td>92.9% (117/126)</td>
<td>96.4% (133/138)</td>
<td>0/27</td>
</tr>
</tbody>
</table>

*Media and supplements purchased in respective countries.
†Etest Pen, penicillin; Spec, spectinomycin; Tet, tetracycline; Ery, erythromycin; Cx, ceftriaxone; Cip, ciprofloxacin; Ce, cefixime; Azi, azithromycin.
§Only one control and three test isolates were available for testing.
¶Only one control and four test isolates were viable for testing.
interpretation agreement ranging from 64.7% to 87.1%. Table 1 outlines the participating laboratories involvement and performance in the programme.

In table 2, MICs and interpretations are compared with their modes for each antibiotic. The percentages of agreement between MIC results and their modes for erythromycin, spectinomycin, cefixime and azithromycin were >90%. Tetracycline, ceftriaxone and ciprofloxacin ranged from 84.3% to 89.1%, while penicillin had 78.8% agreement between MICs and modal MICs. The proportion of agreement between interpretations for all the antibiotics except penicillin and tetracycline was ≤1.5%. Tetracycline has a high percentage of modal MICs (72.7% (16/22)) at interpretative breakpoints which may have contributed to lower agreement between interpretations. It is important to note that there were ≤1.5% false susceptible interpretations for all antibiotics combined.

DISCUSSION
The International Gonococcal Antimicrobial Susceptibility Quality Control Comparison Programme encountered various challenges such as different testing methodologies and media as well as having to ship isolates across great distances to countries with different custom clearance requirements. Despite these challenges and that some of the participants were only able to test a limited number of antimicrobials on a maximum of 18 isolates, most of the participants had acceptable results (>90%).

In the 1990s, this same programme included nine participating laboratories from South America and the Caribbean plus the US Centers for Disease Control and the Canadian Laboratory Centre for Disease Control. They all achieved an overall average agreement with the GASP Coordinating Centre’s results of >90%, ranging from 66.7% to 100% over six panels. Due to lack of sustained funding, this programme was suspended in 2000.

The results from this QC comparison programme were similar to other international programmes. Australia monitors the quality of their antimicrobial resistance data by providing an ongoing external quality assurance programme that was found to have an overall error rate of 3.1% over a 9-year period. The Indian Gonococcal Antimicrobial Surveillance Programme External Quality Assurance schemes for disc diffusion (2001–2007) found an overall interpretation concordance of 82% for five antibiotics and six participating laboratories.

The 10 laboratories participating in the Canadian National Gonococcal Antimicrobial Susceptibility Comparison Programme between 2003 and 2012 also achieved an overall average agreement of ≥90% for MICs and interpretations over 25 panels of five N. gonorrhoeae isolates. These participants had the benefit of using the same media for their testing, although their methods varied between agar dilution and Etest. The European N. gonorrhoeae Antimicrobial Resistance External Quality Assurance Programme provides a panel of 10 N. gonorrhoeae isolates to up to 22 laboratories annually. In 2011 and 2012, participants achieved an overall concordance of ≥90% for both MICs and interpretations. In 2012, MIC concordance for ceftriaxone was found to be less than for other antibiotics at 88.6%, similar to our comparison programme’s 86.3% concordance between ceftriaxone MICs.

Some of the diversity between MICs found in this study could be due to the different testing methods employed. Tetracycline, ceftriaxone and cefixime have been found to have significant (p<0.05) differences between agar dilution MICs and Etest MICs using the matched-pair t-test. While the percentages of concordance for penicillin, tetracycline and ciprofloxacin MICs were below the acceptable parameter (90%), these antibiotics are seldom used to treat gonorrhoea since there is a high possibility of resistance. Ceftriaxone, however, is a drug currently used to treat gonorrhoea and while its MIC concordance was <90%, its categorical interpretation concordance was well above 90% making the discrepancies less likely to have a clinical impact.

Surveillance programmes monitoring N. gonorrhoeae antimicrobial resistance are of upmost importance to provide updated treatment recommendations to mitigate the risk of untreatable gonorrhoea. External quality assurance programmes are essential for surveillance programmes to ensure their data are reliable and can be used effectively. While the results of quality assurance assessment for countries in Latin America are promising, it is important to ensure continuation of the programme to ensure that the participants can identify and correct any problems as they arise and continue to generate reproducible and reliable data. Achieving appropriate levels of agreement between laboratories shows that the antimicrobial susceptibility data generated is accurate and can be used to compare data internationally. Changing levels in antibiotic resistance will be detected properly and treatment guidelines can be updated to successfully manage gonococcal infections.

Key messages
► International Gonococcal Antimicrobial Susceptibility Quality Control Comparison Programme is re-established in Latin America and Caribbean.
► Laboratories from nine countries participated in this programme.
► The participating laboratories had acceptable results, similar to other international quality assurance programmes.

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Acknowledgements We acknowledge Onelkis Feliciano and Oderay Gutiérrez, IPK, Cuba, Ricardo A Giancini, INEI-ANLIS ‘Dr. C. Malbrán’, Buenos Aires, Argentina, and Dr Ana Acevedo Universidad de la Republica, Montevideo, Uruguay, for technical assistance in MIC determination and interpretation of results.

Contributors PG, METC, PAR, OMSC, ALH, MMF, GB, DP, JEM and MB performed MIC testing and collected and reported data. These authors read and offered comments on draft manuscripts. J-ARD established the QC program in Latin America and the Caribbean, worked with IM to develop the protocols and reporting formats used in this study and shipped isolates to participants. J-ARD
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was responsible for the final submission of the manuscript. PS and IM analysed submitted data and drafted the first versions of the manuscript. SDT prepared strains for shipment and co-developed the chocolate agar slant method used to transport isolates. AHG communicated with participants regarding technical questions and comments on the manuscript as well as preparing data summaries for analysis by J-ARD and IM. AHG and SDT also provided comments on versions of the manuscript.

Funding This study was partially funded by the University of Saskatchewan (to JRD) and the Saskatchewan Health Research Foundation (grant no. 9127, Research Alliance for the Prevention of Infectious Disease (RAPID).

Competing interests None declared.

Patient consent Not required.

Ethics approval Not required

Provenance and peer review Not commissioned; externally peer reviewed.

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