Euro-GASP

Annual Report No. 1

2006
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Introduction

Welcome to the first European Gonococcal Antimicrobial Surveillance Programme (Euro-GASP) annual report! Euro-GASP has been established as part of the European Surveillance of Sexually Transmitted Infections (ESSTI) project funded by DG-SANCO.

Surveillance of antimicrobial resistance in Neisseria gonorrhoeae is essential to inform local, national and European guidelines for therapy and prevent the spread of infection. In Europe there are a number of national surveillance programmes but there has been no previous attempt to co-ordinate surveillance of gonococcal antimicrobial resistance across countries in Europe. This is important because there is considerable movement across borders within Europe and comparable data regarding levels of potential therapeutic failure need to be shared to avoid transmission of highly resistant strains.

The ESSTI network is composed of 25 members, which contains a network of 19 laboratories that provide susceptibility data on N. gonorrhoeae. This network will form Euro-GASP by establishing a quality assurance (QA) programme, organisation of training courses, participation in sentinel surveillance studies and molecular typing for outbreak strains. Methodologies used by the Euro-GASP network are presented, along with the network’s current molecular typing activities. The results from the QA panel show some diversity in methodology but good comparability of results. Annual sentinel studies have already highlighted the high levels of resistance to therapeutic agents in Europe and will inform the European guidelines.

A considerable amount has been achieved in the first year of Euro-GASP, and we are confident that the network will go from strength to strength. The success of the network is truly dependent upon the hard work of all the collaborators across Europe, and this report would not have been possible without their efforts.

Professor Catherine Ison, ESSTI project lead.
Michelle Cole, ESSTI Microbiologist
Expansion of the network

This phase of the ESSTI project has seen the expansion of the ESSTI network from 16 members to 25 members. The new members were requested to complete the detailed ESSTI assessment survey describing their STI surveillance programmes and diagnostic and reference laboratory capacity.

Laboratory questionnaire

The ESSTI microbiology network consists of specialist centres for the laboratory diagnosis of *N. gonorrhoeae* in countries previously members of ESSTI (Austria, Belgium, Denmark, England & Wales, Finland, France, Germany, Greece, Ireland, the Netherlands, Norway, Portugal, Scotland, Spain and Sweden) and an additional five members; Estonia, Iceland, Malta, Latvia and Slovenia. The type of reference function of the laboratories was classified as national (the whole country), regional, expert, or other, such as a local diagnostic laboratory. All previous microbiology members completed a cross-sectional survey using a questionnaire to provide information on the laboratory methods for the isolation, identification and susceptibility testing of *N. gonorrhoeae* and these results have been published (1). Recently, this study was expanded by the submission of completed questionnaires from the five new ESSTI microbiology contacts. Estonia submitted questionnaires from two laboratories, so in total there were six completed questionnaires available for analysis.
Isolation and identification of *N. gonorrhoeae*

Of the six laboratories that completed the questionnaire.
- All receive clinical specimens (20 to 150000 per year).
- Three laboratories receive cultures of *N. gonorrhoeae* (100 to 1000 per year).
- Three of the laboratories are referred specimens or isolates from other laboratories (one to seven referring laboratories).
- There are three different types of media used.
There is a wide range of supplements including Polyvitex, yeast autolysate, Gonoline DUO, Vitox, haemoglobin and horse blood. All of the laboratories use selective media, and the antibiotics used for selection are shown below. Oxidase and gram staining is always performed in all laboratories for the identification of *N. gonorrhoeae*, along with at least one other identification procedure.

### Antibiotics used for selection

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colistin</td>
<td>4</td>
</tr>
<tr>
<td>Amphotericin</td>
<td>3</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>3</td>
</tr>
<tr>
<td>Trimetoprim</td>
<td>2</td>
</tr>
<tr>
<td>Lincomycin</td>
<td>1</td>
</tr>
<tr>
<td>Nystatin</td>
<td>1</td>
</tr>
</tbody>
</table>
Tests used for confirming the identity of *N. gonorrhoeae*

<table>
<thead>
<tr>
<th>Test Method</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram staining</td>
<td>6</td>
</tr>
<tr>
<td>Oxidase</td>
<td>6</td>
</tr>
<tr>
<td>Identification kits</td>
<td>5</td>
</tr>
<tr>
<td>Co-agglutination kits</td>
<td>2</td>
</tr>
<tr>
<td>Molecular tests</td>
<td>2</td>
</tr>
</tbody>
</table>

**Susceptibility testing**
All but one of the laboratories performed susceptibility testing.

**Methods used for susceptibility testing**

<table>
<thead>
<tr>
<th>Method of susceptibility testing (5 laboratories)</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Etest</td>
<td>5</td>
</tr>
<tr>
<td>Disc diffusion</td>
<td>3</td>
</tr>
</tbody>
</table>

**Detection of beta-lactamase production (6 laboratories)**

<table>
<thead>
<tr>
<th>Method of detection</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chromogenic cephalosporin nitrocefin</td>
<td>4</td>
</tr>
<tr>
<td>Penicillinase production well in identification kits</td>
<td>2</td>
</tr>
</tbody>
</table>

The reference method used by all laboratories performing susceptibility testing was CLSI (Clinical and Laboratory Standards Institute) and three of the laboratories use *N. gonorrhoeae* strain ATCC 49226 as a control, as well as one laboratory using strain ATCC 43069 also. The most common antimicrobial agents tested across the laboratories are penicillin, ceftriaxone, tetracycline and ciprofloxacin. Five laboratories participate in an external QA programme and only one in a surveillance programme. Of the laboratories that stated they report *N. gonorrhoeae* diagnoses (4/6), three stated that reporting is mandatory and the information is transmitted by paper either monthly or daily. The other laboratory report annually.

By the use of a questionnaire ESSTI has established the methodologies for the identification, isolation and susceptibility testing of *N. gonorrhoeae* in these countries. As shown in the previous questionnaire distribution (1), there is considerable diversity in the services offered for *N. gonorrhoeae* testing,
which could be due to the differences in the prevalence of *N. gonorrhoeae*. There is considerable variation in the number of isolates and cultures received and the number of referring laboratories. All the laboratories identified *N. gonorrhoeae* by a gram staining, oxidase and by the use of at least one other test, which are all appropriate for *N. gonorrhoeae* identification. The most similarities were found in the type of media, the reference method for susceptibility testing and the range of antimicrobials tested. This information is extremely useful for the recommendation of susceptibility testing methods, as the consensus methods used may be the most appropriate for recommendation.

Reference
Laboratory methods for the diagnosis of bacterial sexually transmitted infections across Europe

Introduction
Comparability of surveillance data for sexually transmitted infections (STI) across countries is dependent on the methodology used for diagnosis. In 2003 in order to facilitate comparison of surveillance data obtained across European countries, a questionnaire survey concerning methodologies used for the laboratory diagnosis of bacterial STIs was carried out.

Replies to questionnaire received by country (total =94)

<table>
<thead>
<tr>
<th>Country</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Austria</td>
<td>5</td>
</tr>
<tr>
<td>Greece</td>
<td>5</td>
</tr>
<tr>
<td>Belgium</td>
<td>4</td>
</tr>
<tr>
<td>Ireland</td>
<td>25</td>
</tr>
<tr>
<td>Denmark</td>
<td>5</td>
</tr>
<tr>
<td>Netherlands</td>
<td>1</td>
</tr>
<tr>
<td>England</td>
<td>14</td>
</tr>
<tr>
<td>Norway</td>
<td>2</td>
</tr>
<tr>
<td>Finland</td>
<td>5</td>
</tr>
<tr>
<td>Portugal</td>
<td>8</td>
</tr>
<tr>
<td>France</td>
<td>8</td>
</tr>
<tr>
<td>Scotland</td>
<td>3</td>
</tr>
<tr>
<td>Germany</td>
<td>4</td>
</tr>
<tr>
<td>Spain</td>
<td>2</td>
</tr>
<tr>
<td>Sweden</td>
<td>3</td>
</tr>
</tbody>
</table>

Laboratory diagnosis of gonorrhoea

Location of diagnostic testing for gonorrhoea

(Number of labs)

![Diagram showing location of diagnostic testing for gonorrhoea]
Site or specimen used for diagnosis of gonorrhoea in men
(Number of labs)

Site or specimen used for diagnosis of gonorrhoea in women
(Number of labs)
Isolation and identification of *N. gonorrhoeae*

- Culture medium used for isolation is predominantly commercially produced.
- Most laboratories use a selective medium, but many use a combination of selective and non-selective.
- Antibiotics added include vancomycin, lincomycin, colistin, trimethoprim and amphotericin or nystatin.
- The majority of specimens are inoculated directly onto the medium, mostly using a swab.
- Identification is performed in most laboratories using oxidase, Gram stain and identification kits such as API NH.
- The immunological test (Phadebact test) and carbohydrate utilisation tests are also commonly used.

Method of susceptibility testing for *N. gonorrhoeae*.

(Number of labs)

- Disc (63)
- Breakpoints (1)
- E tests (15)
- MIC (2)
Laboratory diagnosis of chlamydial infection

Site or specimen used for diagnosis of chlamydial infection in men
(Number of labs)

Site or specimen used for diagnosis of chlamydial infection in women
(Number of labs)
Laboratory diagnosis of syphilis

**Diagnosis of primary syphilis**

- Dark ground microscopy
  - 42 centres do test.
  - 34 do not test.
  - (18 centres did not respond).
  - Majority of tests performed near to the patient.

- Molecular methods (PCR)
  - Only 5 labs have access to molecular methods.
  - Only one is a multiplex PCR.

**Screening tests used for detection of antibody in syphilis**

(number of tests)
Summary

- A total of 94 replies were received from 15 countries.

- Microscopy for intracellular Gram negative diplococci and culture for *Neisseria gonorrhoeae* are used in most laboratories for the diagnosis of gonorrhoea.

- Molecular testing is only used infrequently for the diagnosis of gonorrhoea.

- In contrast, chlamydial infection is diagnosed primarily by a molecular test.

- Culture for *Chlamydia trachomatis* has been retained as a diagnostic method by 15 of 94 laboratories.

- Positive tests by molecular testing for chlamydia are often confirmed but usually by the same test.

- The diagnosis of primary syphilis is performed by dark ground microscopy in many laboratories and only occasionally by molecular methods.

- Screening for antibody in syphilis uses a variety of methods including qualitative and quantitative TPHA/TPPA and qualitative VDRL.

- Enzyme immunoassays (EIA) are being used as a screening test in 25 of 94 laboratories but the result is not usually confirmed.

- This survey has for the first time, highlighted the diversity of methods used for the laboratory diagnosis of STIs in Western Europe.
Ciprofloxacin resistant gonorrhoea

Ciprofloxacin remains a recommended therapy for gonorrhoea in Europe despite high levels of resistance in some countries. The results of the first ESSTI sentinel surveillance study in 2004 showed that resistance to ciprofloxacin was over 5% in all countries that participated and showed a mean level of 31% (Martin et al 2006).

Due to a gap between ESSTI projects it was not possible to perform a sentinel study in 2005 and retrospective collection of isolates from 2006 is currently taking place. The ESSTI hub, therefore, requested information on ciprofloxacin resistant gonococci from all collaborative countries using their national surveillance data to provide information on trends in resistance to ciprofloxacin.

Data was available from Austria, Denmark, England & Wales, France, the Netherlands, Scotland and Sweden. The comparison of ciprofloxacin resistance in 2005 and 2006 is shown in figure 1 and for the six countries for which data was available for both years an increase was found in 2006 compared to 2005. Trends in levels of resistance were available for five countries between 2001 and 2006 and in each country resistance increased over time.

This data highlights the need to use ciprofloxacin for the treatment of gonorrhoea with caution and for the need to review the European guidelines.

Data provided by Angelika Stary (Austria), Steen Hoffmann (Denmark), Stephanie Chisholm (England & Wales), Patrice Sendaoui (France), Marianne van der Sande (the Netherlands), Hugh Young and Lesley Wallace (Scotland) and Anders Blaxhult (Sweden).

Reference
Martin IMC, Hoffmann S, Ison CA on behalf of the European Surveillance of Sexually Transmitted Infections (ESSTI) network. European Surveillance of Sexually Transmitted Infections (ESSTI): the first combined antimicrobial susceptibility data for Neisseria gonorrhoeae in Western Europe. J Antimicrob Chemother. 2006;58:587-93
Figure 1: Ciprofloxacin resistance in 2005 and 2006

Figure 2: Ciprofloxacin resistance between 2001-2006
**Gonococcal quality assurance panel (QA2007) distribution results**

A significant aspect of Euro-GASP (the microbiology component of ESSTI) is the annual distribution of a quality assurance (QA) panel. The aim of this annual QA exercise is to identify reproducibility problems within laboratories, offer an overall comparison between the participating laboratories and to explore the comparability of susceptibility data across Europe. Ultimately, the effects of the different methods on concordance of results can be examined, with the aim of clarifying best practice.

The panel consisted of 30 cultures of *N. gonorrhoeae*, which were 10 strains in triplicate. The isolates of the panel were chosen to cover a range of susceptibility to therapeutic antimicrobial agents.

The panel was tested by 19 participating laboratories.

The participating laboratories tested the panel of 30 cultures using their own routine methodology, and the results were returned centrally for analysis. A minimum set of therapeutic antimicrobials were agreed to be tested against the panel; azithromycin, ciprofloxacin, a cephalosporin, penicillin, spectinomycin and tetracycline. Seventeen of the 19 centres chose to test ceftriaxone as their cephalosporin, whereas the other two centres chose to test either cefixime or cefotaxime.
Each centre reported details on their testing methodology used and described the breakpoints for determining the category of resistance (whether a strain is reported as resistant, intermediate or susceptible) for each antimicrobial. For each isolate, the laboratories reported the results as the category of resistance and the MIC or zone of inhibition. Results were decoded and sent back to the laboratories so the centres could study their intra-laboratory reproducibility and start working on any identified problems immediately. Due to the variation in breakpoints and so the different methods could be compared, (zone sizes for disc diffusion and MICs for Etest and agar dilution), the analysis was performed using the category of resistance result. The consensus was established by establishing the most commonly occurring category of resistance.

A range of media was used for susceptibility testing and the most common inoculum size was a suspension equivalent to a 0.5 MacFarland’s standard (approximately $10^4$ cfu/ul).

Most laboratories (11/19) stated that they performed the susceptibility testing in accordance with the Clinical Laboratory Standards Institute (CLSI) guidelines, which include recommended breakpoints. Of those centres that did not state the use of any particular guidelines (5/19), four were using the...
CLSI breakpoints. The remaining three laboratories adhered to local susceptibility testing guidelines, but there was some overlap with the CLSI breakpoints.

Seventeen laboratories were successful in retrieving and testing the panel of 30 cultures, and the remaining two centres retrieved and tested 27 and 28 strains, resulting in a total of 565 strains tested throughout Europe.

Intra-laboratory testing compares the three results for each isolate (the triplicates) and whether the laboratory reported the same category for each isolate three times. The intra-laboratory concordance was high for cephalosporin (100%), spectinomycin (99%), ciprofloxacin (98%) and azithromycin (95%). The lower concordance of the penicillin and tetracycline was most probably due to some strains in the panel being close to breakpoints. This means that even if the three strains in the triplicate were just one dilution different from each other, they may cross over into a different category of resistance. Out of the three susceptibility testing methods, the agar dilution technique demonstrated the highest intra-laboratory concordance.

### Intra-laboratory concordance (%)

<table>
<thead>
<tr>
<th></th>
<th>All methods</th>
<th>Etest</th>
<th>Agar dilution</th>
<th>Disc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cephalosporin</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Spectinomycin</td>
<td>99</td>
<td>98</td>
<td>100</td>
<td>NR*</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>98</td>
<td>98</td>
<td>98</td>
<td>95</td>
</tr>
<tr>
<td>Azithromycin</td>
<td>95</td>
<td>91</td>
<td>98</td>
<td>100</td>
</tr>
<tr>
<td>Penicillin</td>
<td>89</td>
<td>88</td>
<td>94</td>
<td>90</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>86</td>
<td>90</td>
<td>96</td>
<td>70</td>
</tr>
</tbody>
</table>

*NR = No result, as only one laboratory tested the panel against spectinomycin

The overall concordance, which encompasses both intra- and inter-laboratory variation, was highest again for the cephalosporin (100%), spectinomycin (98%) and ciprofloxacin (93%), and lowest for tetracycline (81%). The disc diffusion method gave the highest concordance between the centres (3 to 5 laboratories) that used this method.

### Overall concordance (%)

<table>
<thead>
<tr>
<th></th>
<th>All</th>
<th>Etest</th>
<th>Agar dilution</th>
<th>Disc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cephalosporin</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Spectinomycin</td>
<td>98</td>
<td>98</td>
<td>98</td>
<td>NR*</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>93</td>
<td>93</td>
<td>93</td>
<td>96</td>
</tr>
<tr>
<td>Azithromycin</td>
<td>88</td>
<td>87</td>
<td>93</td>
<td>100</td>
</tr>
<tr>
<td>Penicillin</td>
<td>84</td>
<td>84</td>
<td>89</td>
<td>89</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>81</td>
<td>96</td>
<td>81</td>
<td>69</td>
</tr>
</tbody>
</table>

*NR = No result, as only one laboratory tested the panel against spectinomycin

Detailed analysis of the concordance, the consensus category of resistance and MICs for each of the 10 strains, revealed that lower concordance was demonstrated when the susceptibility of the strains is close to a breakpoint, in particular in the intermediate range. It was not surprising that 100%
concordance was observed for the cephalosporin as resistance was not detected by any laboratory. In addition, the clear cut-off for spectinomycin, with no intermediate range, most probably accounts for the high concordance with this antimicrobial agent. It should be noted that none of the centres that used the disc diffusion method identified any of the strains as having resistance to azithromycin, which may explain the high concordance in this category.

The consensus for the agar dilution and Etest methods always gave the same category of resistance when compared with the overall consensus, and all but one of the consensus MICs of the two test methods were no more than one dilution difference from each other. This demonstrates the high level of comparability between these two methods. There were six instances when the disc diffusion method gave a different category of resistance consensus when compared to the overall consensus, suggesting that it is more difficult to compare the disc diffusion method to methods that produce an actual MIC.

Seventeen laboratories tested for beta-lactamase production and the majority (16/19) used the chromogenic cephalosporin, nitrocefin, and while one laboratory used the penicillinase production well in an identification kit. Of the 17 centres that tested for beta-lactamase production, 16 gave concordant results and just one centre had discordant results.

Even though the use of GC agar base, suspensions equivalent to a 0.5 MacFarland’s standard and adherence to CLSI breakpoints were common features in the methodology used, there is still variation in susceptibility testing methodology across the Euro-GASP countries. Considering this, we believe the overall the concordance is high, with the concordance lowering due to strains that straddle breakpoint. It is however important to include these strains, as the results from the three QA panel distributions will assist in the creation of a quality control (QC) panel. This QC panel will be selected on the basis of the consensus results and should have well defined MIC ranges, so it can be used by individual laboratories to monitor and control their own susceptibility testing. Strains close to the breakpoints are important in the final QC panel so a drift in the MICs can easily be detected if there are any problems with any aspect of the susceptibility testing. Strains that are clearly sensitive with very low MICs and strains that are clearly resistant with high MICs should always be correctly identified, as was found in this distribution.

This is the first step for Euro-GASP in monitoring what methods for susceptibility testing are most commonly used across Europe and which breakpoints are most suitable. These results allow us to compare surveillance data from the members of Euro-GASP with a high level of confidence, as a high level of comparability has been demonstrated. It is also possible to identify deficiencies in the susceptibility testing process requiring more standardisation.

A more detailed analysis of this study is available. Please contact essti@hpa.org.uk to request a copy.
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ESSTI outbreak typing activities

Outbreak investigations can be enhanced by studying the molecular epidemiology of bacterial STI strains alongside available epidemiological data. The identification of particular genotypes, transmission chains and clusters can in turn identify sexual networks, transmission patterns and give us an understanding of the distribution of genotypes throughout Europe.

The following molecular typing methods are available to ESSTI members:

1. **Neisseria gonorrhoeae Multi Antigen Sequence Typing (NG-MAST)**
   NG-MAST is a highly discriminatory molecular typing method to identify clusters of indistinguishable gonococci, which in turn can identify transmission chains and sexual networks and provide information for public health interventions. The technique examines sequence variation in two hypervariable genes (por and tbpB). The por gene encodes the gonococcal outer membrane protein and the tbpB gene encodes beta subunit of the transferring-binding protein. The NG-MAST website (www.ng-mast.net) ensures the method has unambiguous, manageable and shareable data.

2. **Treponema pallidum (Arp / Tpr) typing**
   The molecular subtyping system available for *T. pallidum* subspecies *pallidum* is based on analysis of the arp (acidic repeat protein) gene and the tpr (*T. pallidum* repeat) genes of the tpr subfamily II (tpr E, G and J). The arp gene is amplified by PCR and the number of repeat sequences is determined. The tpr gene is amplified by a nested PCR and the fragment digested by a restriction enzyme. The final molecular type is defined based on two loci (e.g. 14d = 14 repeats in the arp gene and tpr RFLP profile “d”). A database of molecular genotypes
can be established and the distribution of these genotypes can be monitored throughout Europe.

3. LGV – L1, L2 and L3 genotyping
Lymphogranuloma venereum (LGV) is caused by *Chlamydia trachomatis* serovars L1, L2, L3. An LGV specific real-time PCR method first confirms the presence of LGV DNA by the use of primers that target a region on the polymorphic membrane protein (*pmpH*) gene. LGV positive specimens are then genotyped using a nested PCR which amplifies the outer membrane protein I (*ompI*) gene. The fragment is digested using a restriction enzyme to differentiate between the three different serovars of LGV. The *ompI* gene of LGV isolates (serovars L1, L2, and L3) can then be fully sequenced to further discriminate between strains and possibly identify transmission chains and/or networks.

The microbiology team at the ESSTI hub can type strains that have been identified from an unexpected and adverse event. Strains that are submitted for molecular typing must be from an event that has also been submitted through ESSTI_ALERT. The molecular typing of outbreak strains that have been identified along side ESSTI_ALERT may add to outbreak investigations.

**Outbreak typing activities to date:**
- Validation of the syphilis typing
- Development of the LGV *ompI* sequencing
- Development of guidelines for submitting strains for molecular typing
ESSTI microbiology collaborators

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