The BSAC Respiratory Resistance Surveillance Programme

Long-term Surveillance of Antimicrobial Resistance in Respiratory Tract Pathogens

Protocol - version 2.1

22nd March 2006

1 Summary

Study Title:
BSAC Respiratory Resistance Surveillance Programme

Initiator:
British Society for Antimicrobial Chemotherapy.

Funding:
The study will normally be funded by sponsorship from two or more pharmaceutical companies.

Objective:
Determination of the antimicrobial susceptibility of currently circulating lower respiratory tract isolates of *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Moraxella catarrhalis*.

Central Testing Laboratory:
GR Micro Limited, London

Geographical Scope:
21 collecting centres have been selected to give good geographical spread throughout the United Kingdom and Ireland.

Selection and Numbers of Isolates:
The organisms to be collected are *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Moraxella catarrhalis* from the lower respiratory tract of patients with suspected community-acquired lower respiratory tract infection, excluding repeat isolates from the same episode of infection and patients with cystic fibrosis.

The period of collection each winter season will be:
1st October – 30th April

Each centre will collect up to:
50 consecutive isolates of *S. pneumoniae*
50 consecutive isolates of *H. influenzae*
25 consecutive isolates of *M. catarrhalis*

The total number of isolates collected each year will be up to:
1000 *S. pneumoniae*
1000 *H. influenzae*
500 *M. catarrhalis*

Testing of Isolates:
The isolates will be re-identified by the Central Testing Laboratory and tested using the BSAC agar dilution method for determination of minimum inhibitory concentration.

2 Selection, Collection and Transportation of Isolates

2.1 Selection of Isolates

Each collecting centre will collect up to 50 consecutive isolates of *Streptococcus pneumoniae*, 50 consecutive isolates of *Haemophilus influenzae* and 25 consecutive isolates of *Moraxella catarrhalis* meeting the following criteria.

Inclusion criteria:

1 isolates from lower respiratory tract samples
2 patients with presumed lower respiratory tract infection
3 between 1 October and 30 April each winter.

Exclusion criteria:

1 patients who have been in hospital more than 48 hours at the time of sample collection
2 patients with cystic fibrosis
3 repeat isolates from the same infection episode i.e. isolates taken within two weeks of previous lower respiratory tract isolate from the same patient

2.2 Storage of Isolates in Collecting Laboratories

Isolates will be stored frozen using the Microbank™ bead storage cryovial system according to the manufacturer's instructions, or another suitable storage method.

2.3 Preparation and Transportation of Isolates

Thawed isolates will be subcultured onto non-selective medium to give luxuriant growth after overnight incubation, suspended in transport medium, and sent to the Central Testing Laboratory in compliance with current transport regulations.

3 Additional Data to be supplied with Isolates

For each isolate, the following information will be supplied by the collecting laboratory:

- Date of specimen collection
- Age of patient
- Sex of patient
- Care setting of patient: community (GP), nursing home, hospital (outpatient or inpatient <48 hours)
- Specimen type e.g. sputum, bronchoalveolar lavage, etc.
- Identification of isolate by genus and species
- The collecting laboratory's own antimicrobial susceptibility test results, as available.

4 Identification and Storage of Isolates

On receipt at the Central Testing Laboratory, the isolates will be subcultured on non-selective medium and checked for purity. The isolates will be identified by colonial morphology, microscopy and specific tests as listed below.

Streptococcus pneumoniae
Gram-positive diplococci, growing as alpha-haemolytic sometimes umbonate or mucoid colonies on horse blood agar. Catalase negative with a positive optochin test or positive bile solubility test.

Haemophilus influenzae
Gram-negative coccobacilli, requiring a combination of X (haematin) and V (NAD) factor when grown on a non-supplemented medium. (N.B. *H. parainfluenzae*, requiring factor V only, are excluded.)

Moraxella catarrhalis
Gram negative diplococci, producing entire whitish/grey colonies on horse blood or chocolated horse blood agar. Oxidase positive, butyrate esterase positive.

Samples will be frozen in horse serum at –70°C.

5 Sensitivity Testing of Isolates

Minimum inhibitory concentrations will be measured by the BSAC agar dilution method, summarised in the table below.

[Andrews, J. M. (2001). Determination of minimum inhibitory concentrations. *Journal of Antimicrobial Chemotherapy* **48 Suppl. S1***,* 5-16].

Future BSAC amendments to the original descriptions may be incorporated.

Organism	Medium	Supplements	Atmosphere	Temperature & duration
H. influenzae	Iso-Sensitest agar	5% whole horse blood +20mg/L NAD	air plus 4 - 6% CO_2	35 - 37°C 18 - 20 hours
M. catarrhalis	Iso-Sensitest agar	5% whole horse blood	air	35 - 37°C 18 - 20 hours
S. pneumoniae	Iso-Sensitest agar	5% whole horse blood	air plus 4 - 6% CO_2	35 - 37°C 18 - 20 hours

The inoculum size is 10^4 CFU/spot, except for *M. catarrhalis* tested against β-lactams, which require 10^6 CFU/spot.

Isolates of *Haemophilus influenzae* and *Moraxella catarrhalis* will also be tested for β-lactamase activity (by nitrocefin test) and for reduced susceptibility to quinolones (using 30μg nalidixic acid disc).

6 Antimicrobial Agents for Testing, and Testing Ranges

The isolates will be tested against a range of antimicrobial agents. The tests and agents listed below form the 'continuity group' and are intended to be studied for the full term of the programme. Additional agents will be tested in some seasons.

The concentration ranges shown are the planned initial testing ranges. All organisms inhibited throughout the range will be retested with lower concentrations to establish the exact MIC, and no MICs will be reported as 'less than or equal to'. Generally, isolates not inhibited by the highest initially-tested concentration will be re-tested with higher concentrations to establish the exact MIC, but this process may be limited by practical considerations including the solubility of the compound. Exceptions are clindamycin, erythromycin, tetracycline and trimethoprim for *S. pneumoniae*, and tetracycline for *H. influenzae* and *M. catarrhalis*: censored MICs for these agents will be initially listed as 'greater than the highest tested concentration', which may be translated to 'greater than or equal to twice the highest tested concentration' in published tables.

S. pneumoniae		
Agent or test	Initial range, mg/L	High MICs censored at
penicillin	0.008 - 4	not censored
amoxicillin	0.008 - 4	not censored
cefuroxime	0.015 - 8	not censored
cefotaxime	0.008 - 4	not censored
ciprofloxacin	0.12 - 16	not censored
erythromycin	0.015 - 16	>16
clindamycin	0.06 - 2	>2
tetracycline	0.03 - 16	>16
trimethoprim	0.03 - 32	>32

H. influenzae		
Agent or test	Initial range, mg/L	High MICs censored at
β-lactamase[1]	n/a	n/a
nalidixic acid[2]	n/a	n/a
ampicillin	0.06 - 8	not censored
amoxicillin	0.06 - 16	not censored
amoxicillin-clavulanate[3]	0.06 - 8	not censored
cefuroxime	0.12 - 32	not censored
cefotaxime	0.004 - 1	not censored
ciprofloxacin	0.004 - 4	not censored
erythromycin	0.03 - 64	not censored
tetracycline	0.03 - 8	>8
trimethoprim	0.015 - 16	not censored

M. catarrhalis		
Agent or test	Initial range, mg/L	High MICs censored at
β-lactamase[1]	n/a	n/a
nalidixic acid[2]	n/a	n/a
amoxicillin-clavulanate[3]	0.06 - 8	not censored
cefuroxime	0.12 - 32	not censored
ciprofloxacin	0.004 - 4	not censored
erythromycin	0.03 - 64	not censored
tetracycline	0.03 - 8	>8

[1] Test for β-lactamase using the chromogenic cephalosporin nitrocefin.
[2] Test for quinolone resistance using 30µg nalidixic acid disc.
[3] Test using 2:1 ratio amoxicillin:clavulanic acid; reported concentrations refer to amoxicillin.

7 Quality Assurance

Ten isolates of known antimicrobial sensitivity will be supplied to the Central Testing Laboratory by an external laboratory for testing by the methods of this programme each season.

8 Data Handling

There will be suitable safeguards to ensure that data are entered into the study records accurately, maintained securely, and disseminated only to authorised recipients.
The complete data and summaries will be supplied to the BSAC by the Central Testing Laboratory by 30th June each year.
The complete data for each testing season will include a listing to show, for each isolate, the background information (patient age etc.) and MIC for each agent tested.
The data for each season will be summarised to show, as a minimum, the MIC range, MIC_{50} and MIC_{90} for each species and antimicrobial agent.
Information on additional agents tested in the programme may be confidential to a sponsoring company; confidential information will be seen by staff closely involved with the surveillance programme at the Central Testing Laboratory and the BSAC, but not included in listings for collecting laboratories or other sponsoring companies.
Information under the control of the BSAC (continuity group tests and information on sponsored agents where the sponsor has allocated control to the BSAC) will be widely disseminated. It will be circulated in detailed form (line-listed by isolate) to all full sponsors for that season, and it will be made available in suitable summary form for at least a year through a website. Each season, data under the control of the BSAC will be supplied to each collecting laboratory showing the line-listed results for the isolates supplied by that laboratory.
If isolates are to be categorised as susceptible/intermediate/resistant, the BSAC breakpoints current at the time (www.bsac.org.uk) will be used.

9 Collecting Laboratories

The collecting laboratories are selected to give good geographical coverage of the United Kingdom and Ireland, with a range of catchments (urban/rural, teaching/non-teaching hospitals, more/less socially deprived). In some cases, two laboratories serving the same region are combined to contribute one quota of isolates. If a laboratory withdraws from the programme, it will be replaced if possible with one or more laboratories serving an area nearby.

The twenty-one centres currently contributing (2005-06) are:

Collecting Laboratory	Town/City	Country
Manor Hospital Walsall	Birmingham / West Midlands	England
New Cross Hospital Wolverhampton	Birmingham / West Midlands	England
Southmead Hospital	Bristol	England
Addenbrooke's Hospital	Cambridge	England
Leeds General Infirmary	Leeds	England
Leicester Royal Infirmary	Leicester	England
University of Liverpool	Liverpool	England
St Bart's & Royal London School of Medicine	London	England
University College Hospital London	London	England
Hope Hospital	Manchester	England
Derriford Hospital	Plymouth	England
Southampton General Hospital	Southampton	England
Sunderland Royal Infirmary	Sunderland	England
St Vincent's Hospital and Beaumont Hospital	Dublin	Ireland
University College Hospital Galway	Galway	Ireland
The Royal Hospitals and Ulster Hospital	Belfast & Dundonald	N. Ireland
Aberdeen Royal Infirmary	Aberdeen	Scotland
New Royal Infirmary	Edinburgh	Scotland
Glasgow Royal Infirmary	Glasgow	Scotland
University Hospital of Wales	Cardiff	Wales
Wrexham Maelor Hospital	Wrexham	Wales

10 Protocol Amendments - past and future

10.1 Future Amendments

Amendments to this protocol can be made by agreement of the BSAC Extended Working Party on Respiratory Resistance Surveillance.

10.2 Past Differences

The BSAC Respiratory Resistance Surveillance Programme has run since the winter of 1999-2000, based on an original protocol dated 7 February 2000. The following differences from the current protocol have existed at different times.

Antimicrobials tested

Up to and including 2004-05, cefaclor was tested against all three species.
Up to and including 2004-05, the list of agents tested against *M. catarrhalis* was the same as for *H. influenzae*, so included ampicillin, amoxicillin, cefaclor, cefotaxime and trimethoprim.

Ranges and censoring

In the first season, 1999-2000, high MICs for a few isolates were censored where in later years they would have been re-tested to give an exact value. Conversely, clindamycin, erythromycin and tetracycline were tested at high concentration levels that would now be censored.

M. catarrhalis - missing data

In the first two years of the programme, there was uncertainty as to the correct inoculum for testing *M. catarrhalis* with β-lactam antimicrobials. Some were tested at both 10^4 and 10^6 CFU/spot, but not all. Results for amoxicillin, cefaclor, cefuroxime and cefotaxime at the now-recommended 10^6 CFU/spot are therefore not available for 1999-2000. Results with 10^4 CFU/spot are no longer published on the website, but are available if required.
Owing to budgetary constraints, *M. catarrhalis* were collected (with background information) and stored but not tested against the full range of antimicrobials in 2001-02 and 2003-04.

Care settings

Information was collected separately for outpatients and inpatients (less that 48 hours) in 1999-2000; the categories have been combined since 2000-2001.

Changes to Collecting Laboratories

Until 2005-06, there were twenty collecting centres. This increased to twenty-one with the replacement of Birmingham City Hospital by Manor Hospital, Walsall and New Cross Hospital, Wolverhampton.

Now	Previously
St. Vincent's Hospital and Beaumont Hospital, Dublin. From 2001-2002.	Meath Adelaide and Royal Children's Hospital, Dublin. 1999-2001
The Royal Hospitals, Belfast, and Ulster Hospital, Dundonald. From 2001-2002.	The Royal Hospitals Belfast (alone). 1999-2001
Queen Elizabeth Hospital, Gateshead From 2002-2003.	Freeman Hospital, Newcastle. 1999-2002
New Royal Infirmary, Edinburgh. (Nominal change only, due to laboratory relocation.) From 2002-03.	Western General Hospital, Edinburgh. 1999-2002
Glasgow Royal Infirmary From 2003-2004.	Southern General Hospital, Glasgow 1999-2003
Sunderland Royal Infirmary, Sunderland From 2004-2005.	Royal Victoria Hospital, Newcastle 1999-2001 Freeman Hospital, Newcastle 2001-2002 Queen Elizabeth Hospital, Gateshead 2002-04
Manor Hospital, Walsall From 2005-06.	Birmingham City Hospital 1999-2005
New Cross Hospital, Wolverhampton From 2005-06.	Birmingham City Hospital 1999-2005
Leeds General Infirmary, Leeds From 2005-06	St. James' University Hospital, Leeds 1999-2005