

The BSAC Respiratory Resistance Surveillance Programme

Long-term Surveillance of Antimicrobial Resistance in Respiratory Tract Pathogens

Protocol - version 3.3.1

1st December 2009

applies to isolates collected October 2009 - September 2010

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1 Summary

Study Title:

BSAC Respiratory Resistance Surveillance Programme

Initiator:

British Society for Antimicrobial Chemotherapy.

Funding:

The study will normally be funded by contributions from two or more pharmaceutical companies.

Objective:

Determination of the antimicrobial susceptibility of currently circulating lower respiratory tract isolates of community-acquired *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Moraxella catarrhalis*, and hospital-acquired *Staphylococcus aureus*, *Pseudomonas* spp., *Acinetobacter* spp., *Escherichia coli*, *Klebsiella* spp. and *Enterobacter* spp.

Central Testing Laboratory:

Quotient Bioresearch Limited, Microbiology

Geographical Scope:

At least 20 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 tested are *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Moraxella catarrhalis* from the lower respiratory tract of patients with suspected **community-acquired lower respiratory tract infection**, as well as isolates of *Staphylococcus aureus*, *Pseudomonas* spp., *Acinetobacter* spp., *Escherichia coli*, *Klebsiella* spp. and *Enterobacter* spp. from **hospital-acquired lower respiratory tract infection**, excluding repeat isolates from the same episode of infection and patients with cystic fibrosis.

The period of collection each season will be:

1st October – 30th September

Each centre will collect from **community-acquired lower respiratory tract infections** up to:

25 consecutive isolates of *S. pneumoniae*

25 consecutive isolates of *H. influenzae*

13 consecutive isolates of *M. catarrhalis*

And from **hospital-acquired lower respiratory tract infections** up to:

13 consecutive isolates of *S. aureus*

13 consecutive isolates of *Pseudomonas* spp.

13 consecutive isolates of *Acinetobacter* spp.

50 consecutive isolates of Enterobacteriaceae

The target total number of isolates to be collected each season will be:

500 *S. pneumoniae*

500 *H. influenzae*

260 *M. catarrhalis*

260 *S. aureus*

260 *Pseudomonas* spp.

260 *Acinetobacter* spp.

1000 Enterobacteriaceae*

* The Enterobacteriaceae will be collected as unspicated 'coliforms' and identified to genus (and normally to species) level centrally. *E.coli*, *Klebsiella* spp. and *Enterobacter* spp. will be accepted into the study; other species will be discarded. Hence, the total number to be fully tested will be less than 1000.

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

Community-acquired infection

Each collecting centre will collect up to 25 consecutive isolates of *Streptococcus pneumoniae*, 25 consecutive isolates of *Haemophilus influenzae* and 13 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 collected between 1 October and 30 September.

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 of the same species taken within two weeks of previous lower respiratory tract isolate from the same patient

Hospital-acquired infection

Each collecting centre will collect up to 13 consecutive isolates of *Staphylococcus aureus*, 13 consecutive isolates of *Pseudomonas* spp., 13 consecutive isolates of *Acinetobacter* spp. and up to 50 consecutive isolates of Enterobacteriaceae meeting the following criteria:

Inclusion criteria:

- 1 isolates from lower respiratory tract samples
- 2 patients with presumed lower respiratory tract infection
- 3 collected between 1 October and 30 September.
- 4 judged clinically significant by the responsible medical microbiologist
- 5 patient in hospital 48 hours or longer at the time of collection of the first positive sample.

Exclusion criteria:

- 1 patients with cystic fibrosis
- 2 repeat isolates from the same infection episode i.e. isolates of the same species 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 sub-cultured 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

Community-acquired infection

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.

Hospital-acquired infection

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

- Date of specimen collection
- Age of patient
- Sex of patient
- Confirmation that patient has been in hospital ≥ 48 hours
- Time in hospital before sample collection, if known.
- Ventilator status – ventilated/ not ventilated/ ventilation status unknown
- Speciality – A & E, cardiology, care of the elderly, general medicine, haematology/oncology, high dependency unit, intensive care unit, nephrology/renal unit, paediatrics, surgery, other, unknown.
- Specimen type e.g. sputum, bronchoalveolar lavage, etc.
- Identification of isolate by genus and species, if known.
- 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.

Community-acquired infection

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 chocolate horse blood agar. Oxidase positive, butyrate esterase positive.

Hospital-acquired infection

Staphylococcus aureus

Gram-positive cocci, producing white or yellow colonies on horse blood or chocolate horse blood agar. Catalase positive, positive agglutination with Staphylococcal latex, DNase production.

Pseudomonas spp.

Gram-negative bacilli, oxidase positive, identified to species level using the bioMérieux Analytical Profile Index (API) identification method 20NE.

Acinetobacter spp.

Gram-negative bacilli, oxidase negative, identified to species level using the bioMérieux Analytical Profile Index (API) identification method 20NE.

Enterobacteriaceae

Gram-negative bacilli, catalase positive, oxidase negative, identified to species level by using the bioMérieux Analytical Profile Index (API) identification method 20E.

Organisms will be identified to species level in the large majority of cases, with occasional exceptions for particularly unusual species, which may be reported as genus spp.

Isolates of *E. coli*, *Klebsiella* and *Enterobacter* will be tested for antimicrobial susceptibility. Other hospital-acquired isolates of Enterobacteriaceae will be discarded without further testing.

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	Spot size (CFU/mL)	Atmosphere	Temperature & duration
<i>H. influenzae</i>	Iso-Sensitest agar	5% defibrinated horse blood +20mg/L NAD	10 ⁴	air plus 4 - 6% CO ₂	35 - 37°C 18 - 20 hours
<i>M. catarrhalis</i>	Iso-Sensitest agar	5% defibrinated horse blood	10 ⁴ (10 ⁶ against β -lactams)	air	35 - 37°C 18 - 20 hours
<i>S. pneumoniae</i>	Iso-Sensitest agar	5% defibrinated horse blood	10 ⁴	air plus 4 - 6% CO ₂	35 - 37°C 18 - 20 hours
<i>S. aureus</i> (tests other than oxacillin)	Iso-Sensitest agar	None	10 ⁴ (10 ⁶ against penicillin)	air	35 - 37°C 18 - 20 hours
<i>S. aureus</i> (oxacillin)	Columbia agar	2% NaCl	10 ⁴	air	30°C 24 hours
<i>Enterobacteriaceae</i>	Iso-Sensitest agar	None	10 ⁴	air	35 - 37°C 18 - 20 hours
<i>Pseudomonas spp.</i>	Iso-Sensitest agar	None	10 ⁴	air	35 - 37°C 18 - 20 hours
<i>Acinetobacter spp.</i>	Iso-Sensitest agar	None	10 ⁴	air	35 - 37°C 18 - 20 hours

Special conditions may apply for other antimicrobials not included in the continuity group, for example Ca²⁺-supplemented isotonic medium for daptomycin.

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. In some cases (shown in brackets in the table) extended ranges will be tested if the initial range does not identify the MIC exactly. The ranges are intended to be wide enough to give full endpoints in almost all cases. If not, MICs censored at the upper end of the range will be listed initially 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. MICs censored at the lower end of the range will be listed as 'less than or equal to the lowest tested concentration'.

Community-acquired lower respiratory tract infections

Gram-positive isolates

Agent or test	<i>S. pneumoniae</i>
amoxicillin	0.008 - 4 (0, ∞)
cefotaxime	0.008 - 4 (0, ∞)
cefuroxime	0.015 - 8 (0, ∞)
ciprofloxacin	0.12 - 16 (0, ∞)
clindamycin	0.06 - 2 (0)
erythromycin	0.015 - 16 (0)
penicillin	0.008 - 4 (0, ∞)
tetracycline	0.03 - 16 (0)
trimethoprim	0.03 - 32 (0)

Gram-negative isolates

Agent or test	<i>H. influenzae</i>	<i>M. catarrhalis</i>
β -lactamase ¹	n/a	n/a
nalidixic acid ²	n/a	n/a
amoxicillin	0.06 - 16 (0, ∞)	
amoxicillin-clavulanate ³	0.06 - 8 (0, ∞)	0.06 - 8 (0, ∞)
ampicillin	0.06 - 8 (0, ∞)	
cefotaxime	0.004 - 1 (0, ∞)	
cefuroxime	0.12 - 32 (0, ∞)	0.12 - 32 (0, ∞)
ciprofloxacin	0.004 - 4 (0, ∞)	0.004 - 4 (0, ∞)
erythromycin	0.03 - 64 (0, ∞)	0.03 - 64 (0, ∞)
tetracycline	0.03 - 8 (0)	0.03 - 8 (0)
trimethoprim	0.015 - 16 (0, ∞)	

Hospital-acquired lower respiratory tract infections

Gram-positive isolates

DRUG	<i>S. aureus</i>
ciprofloxacin	0.03 - 128
clindamycin	0.03 - 128
erythromycin	0.12 - 128
fusidic acid	0.015 - 256
gentamicin	0.008 - 128
mupirocin	0.06 - 1024
oxacillin	0.03 - 128
penicillin	0.015 - 64
piperacillin- tazobactam ^{4,5}	0.12 - 128
rifampicin	0.004 - 2
teicoplanin	0.06 - 16
tetracycline	0.06 - 128
trimethoprim	0.06 - 128
vancomycin	0.5 - 16

Gram-negative isolates

DRUG	Enterobacteriaceae	<i>Pseudomonas</i>	<i>Acinetobacter</i>
amoxicillin	0.25 - 256		
amoxicillin-clavulanate ³	0.12 - 64		
cefotaxime	0.008 - 16 (256)		
cefoxitin	0.5 - 128		
ceftazidime	0.008 - 16 (256)	0.03 - 16 (256)	0.03 - 16 (256)
cefuroxime	0.12 - 128		
ciprofloxacin	0.008 - 16 (256)	0.002 - 16 (256)	0.002 - 16 (256)
gentamicin	0.12 - 16 (256)	0.12 - 16 (256)	0.12 - 16 (256)
imipenem ⁴	0.008 - 16 (256)	0.03 - 16 (0.008, 256)	0.03 - 16 (0.008, 256)
piperacillin- tazobactam ^{4,5}	0.015 - 256	0.12 - 64 (*256)	0.12 - 64 (*256)
tetracycline	0.12 - 128		0.12 - 128

¹ Test for β -lactamase using the chromogenic cephalosporin nitrocefin.

² Test for quinolone resistance using 30 μ g nalidixic acid disc.

³ Test using 2:1 ratio amoxicillin:clavulanic acid; reported concentrations refer to amoxicillin.

⁴ Poor stability – plates containing these agents to be used on day of preparation.

⁵ Test with tazobactam at 4 mg/L fixed concentration; reported concentrations refer to piperacillin.

(0) unlimited lower level.

(∞) unlimited higher level (within the bounds of solubility and practicality).

10 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-three centres currently contributing (2009-10) are:

Collecting Laboratory	Town/City	Country
North Devon District Hospital	Barnstaple	England
City Hospital	Birmingham	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
Royal London Hospital	London	England
Chelsea and Westminster Hospital	London	England
Salford Royal Hospital	Manchester	England
Southampton General Hospital	Southampton	England
Sunderland Royal Hospital	Sunderland	England
Royal Cornwall Hospital	Truro	England
St Vincent's Hospital and Beaumont Hospital	Dublin (2 half centres)	Ireland
University College Hospital Galway	Galway	Ireland
Ulster Hospital	Belfast	N. Ireland
Aberdeen Royal Infirmary	Aberdeen	Scotland
Royal Infirmary	Edinburgh	Scotland
Southern General Hospital	Glasgow	Scotland
Wishaw General Hospital	Wishaw	Scotland
University Hospital of Wales	Cardiff	Wales
Wrexham Maelor Hospital	Wrexham	Wales

11 Protocol Amendments - past and future

11.1 Future Amendments

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

11.2 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. Up to and including 2007-08, the programme included only community-acquired infections. The last protocol describing surveillance solely of community-acquired infections was version 2.1, 22nd March 2006; it records amendments made between 1999-2000 and 2007-08.

From 2008-09, the surveillance was extended substantially to include hospital-acquired infections. The first protocol describing the extended programme was version 3.1, 9th October 2008.

Changes for version 3.3.1, 1st December 2009 (compared with version 3.2):

- Piperacillin-tazobactam added to the continuity test panel for hospital-acquired isolates. (Piperacillin-tazobactam was previously tested as an additional agent.)
- Collecting laboratories (number increased to 23):
 - North Devon District Hospital added.
 - Hope Hospital, Manchester, renamed as Royal Salford.

Changes for version 3.2, 21st January 2009 (compared with version 3.1):

- Added note of collection period to which the protocol refers.
- Added note that special testing conditions may apply for antimicrobials outside the continuity group (not listed in protocol).

- Added clarification of how MICs are to be recorded following re-testing of cephalosporins for the purpose of ESBL testing.
- ESBL status will be recorded as negative (i.e. not confirmed positive) rather than unknown for isolates showing raised cephalosporin MICs in original testing that are not confirmed at re-test.