

The BSAC Bacteraemia Resistance Surveillance Programme

Long-term Surveillance of the *in vitro* Activity of a Range of Antimicrobial Agents
Against Potential Pathogens Isolated from Blood Samples
of Patients with Clinically Significant Bacteraemia.

Protocol version 2.7

5th February 2010

applies to isolates collected in 2009

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1 Summary

Study Title:

BSAC Bacteraemia 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 bacterial isolates from clinically significant bacteraemia.

Central Testing Laboratory:

Health Protection Agency, Colindale, London.

Geographical Scope:

25 collecting centres have been selected to give good geographical spread throughout the United Kingdom and Ireland.

Selection and Numbers of Isolates

Twelve groups of organisms (six Gram-negative, six Gram-positive) will be collected from the blood of patients with clinically significant bacteraemia, excluding repeat isolates from the same episode of infection. The groups include those organisms ranked as the ten commonest agents of bacteraemia in all of the three years for which information was available prior to the initiation of the study in 2001 (1997-99, HPA LabBase data).

The period of collection will be the calendar year, 1st January to 31st December.

Each centre will collect up to 10 consecutive isolates in each group of organisms (20 of *S. aureus* and *E. coli*), giving a total of up to 250 isolates in each group (500 of *S. aureus* and *E. coli*).

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 Transport of Isolates

2.1 Population

The population for study is all patients with infections giving rise to clinically significant bacteraemia.

2.2 Organisms

The organisms for study are those listed in the table below, when isolated from blood and considered by the responsible medical microbiologist in the collecting institution to be clinically significant.

Gram positive	Gram negative
<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>
coagulase negative staphylococci	<i>Klebsiella spp.</i>
<i>Streptococcus pneumoniae</i>	Proteaeae
other α - & non- haemolytic streptococci	<i>Pseudomonas spp.</i>
β -haemolytic streptococci	<i>Enterobacter spp.</i>
<i>Enterococcus spp.</i>	<i>Serratia spp.</i>

Exclusions:

- 1 Repeat isolates taken within two weeks of a previous clinically significant bacteraemia.

Misidentified and over-quota isolates, and mixed cultures

- 1 Isolates submitted and confirmed as Enterobacteriaceae, but with differing species identification between the central and collecting laboratory, will be included in the study under their central laboratory identification, provided that this does not bring the total of isolates in that organism group from that collecting laboratory over the quota of twenty for *E. coli* and ten for other groups. However, where identification discrepancies involve groups other than Enterobacteriaceae, the isolate will be discarded, and, if time remains in the collecting season, a replacement sought.
- 2 If a collecting laboratory submits more than the quota of ten or twenty isolates of a defined collecting group, excess isolates will be excluded starting with any that were submitted under other names and then by date of collection (latest first).
- 3 In cases of mild, obvious and understandable contamination e.g. an isolate predominantly of *E. coli* with a small number of coagulase-negative staphylococci, attempts will be made to re-isolate and include the primary organism e.g. *E. coli*. Cultures that are grossly mixed, or that are mixtures of organisms from the same group, will be discarded and, if time remains in the collecting season, a replacement will be sought.

2.3 Transport

Collecting laboratories will send isolates to the Central Testing Laboratory on nutrient agar slopes or, in the case of streptococci, on Dorset Egg slopes in compliance with prevailing 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, from the following categories:
 - community (GP) or outpatient
 - hospital inpatient (≤ 48 hours from admission)
 - hospital outpatient (> 48 hours from admission)
- Tentative origin of organism / focus of infection, from the following categories:
 - lines and devices (excluding urinary catheters)
 - genitourinary system (including urinary catheters)
 - respiratory tract
 - gastrointestinal / intra-abdominal
 - skin and soft tissue (including wounds but excluding surgical site wounds)
 - surgical site wounds
 - endocarditis
 - cerebro-spinal fluid
 - other

- For hospital patients, the requesting speciality, from the following categories:
 - intensive care unit / high dependency unit
 - haematology / oncology
 - surgical
 - medical
 - care of the elderly
 - paediatrics
 - nephrology / renal unit
 - cardiology
 - other
- Identification of isolate by genus and species
- The collecting laboratory's own antimicrobial susceptibility test results, as available.

The Central Testing Laboratory will also gather information from each collecting laboratory about other clinically significant isolates from bacteraemia during the year. At minimum, this information will include for each genus and species the total number collected by that laboratory, excluding repeat isolates within two weeks.

4 Identification and Storage of Isolates

On receipt at the Central Testing Laboratory, isolates will be subcultured on appropriate agar to confirm purity and identified to species level as indicated below:

Gram-positive

- Staphylococci, by coagulase tests.
- Pneumococci, optochin sensitivity and serotyping.
- Other α - and non-haemolytic streptococci, with ID32 STREP kits and additional biochemical tests for definitive speciation.
- β -Haemolytic streptococci, Lancefield group.
- Enterococci, by PCR and biochemical tests.

Gram-negative

- Enterobacteriaceae, with API20E strips. *Exception:* isolates identified as *E. coli* by collecting laboratories and giving a pink colour on UTI medium are accepted as *E. coli* without further testing.
- Non-fermenters, with API20NE or API20 strips. *Exception:* isolates identified as *Pseudomonas* by collecting laboratories and giving a blue/green colour on Kings medium are accepted as *Pseudomonas aeruginosa* without further testing.

The identity of unusual isolates will be reconfirmed by Molecular Identification Services at the NCTC. Isolates not conforming to the criteria of section 2 will be discarded without testing.

Isolates will be stored in blood glycerol broth at -70°C.

5 Sensitivity Testing of Isolates

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

Culture Conditions - Gram Negative Organisms

Organisms	medium	supplements	spot size	atmosphere	temperature	incubation time
<i>E.coli</i> , <i>Klebsiella</i> , <i>Enterobacter</i> , non-swarming Proteeae, <i>Serratia</i> , <i>Pseudomonas</i>	Iso-sensitest agar	-	10 ⁴	air	35 - 37°C	18 - 20 hours
swarming Proteeae e.g. <i>Proteus</i> spp.	Iso-sensitest agar	50 g/L PNPG	10 ⁴	air	35 - 37°C	18 - 20 hours

Culture Conditions - Gram Positive Organisms

Staphylococci but not with methicillin, oxacillin, penicillin	Iso-sensitest agar	-	10 ⁴	air	35 - 37°C	18 - 20 hours
<i>Enterococcus</i> spp.	Iso-sensitest agar	-	10 ⁴	air	35 - 37°C	18 - 20 hours
<i>S. pneumoniae</i> & other α- & non-haemolytic streptococci	Iso-sensitest agar	5% defibrinated horse blood	10 ⁴	air + 4-6% CO ₂	35 - 37°C	18 - 20 hours
β-haemolytic streptococci	Iso-sensitest agar	5% defibrinated horse blood	10 ⁴	air	35 - 37°C	18 - 20 hours

Culture Conditions - Special Cases (Gram-negative and Gram-positive) Change from usual conditions for that species shown in bold.

organisms	special case	medium	supplements	spot size	atmosphere	temperature	incubation time
Staphylococci	methicillin oxacillin	Columbia agar	2% NaCl	10 ⁴	air	30°C	24 hours
Staphylococci	penicillin	Iso-sensitest agar	-	10⁶	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 years.

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'.

Gram-positive isolates - staphylococci and enterococci

DRUG	Staphylococci mg/L	Enterococci mg/L
ampicillin		0.03 - 128
ciprofloxacin	0.03 - 128	0.06 - 128
clindamycin	0.03 - 128	
erythromycin	0.12 - 128	0.03 - 256
fusidic acid	0.015 - 256	
gentamicin	0.008 - 128	0.5 - 2048
imipenem ¹		0.06 - 128
mupirocin	0.06 - 1024	
oxacillin	0.03 - 128	
penicillin	0.015 - 64	0.12 - 256
piperacillin-tazobactam ^{1,2} (4mg/L taz)	0.12 - 128 pip + 4 taz	0.03 - 128 pip + 4 taz
rifampicin	0.004 - 2 (ST) 0.004 - 256 (CN)	
teicoplanin	0.06 - 16	0.015 - 128
tetracycline	0.06 - 128	0.12 - 128
trimethoprim	0.06 - 128	
vancomycin	0.5 - 16	0.12 - 128

Gram-positive isolates - streptococci

DRUG	<i>S. pneumoniae</i> mg/L	other α - & non- haemolytic streptococci mg/L	β -Haemolytic streptococci mg/L
amoxicillin	0.004 - 16	0.004 - 16	0.004 - 1
cefotaxime	0.004 - 4	0.004 - 4	0.002 - 1
ciprofloxacin	0.25 - 128	0.25 - 128	0.06 - 128
clindamycin	0.015 - 128	0.015 - 128	0.008 - 128
erythromycin	0.015 - 128	0.015 - 128	0.015 - 128
gentamicin	0.06 - 128	0.06 - 128	0.06 - 128
imipenem ¹	0.002 - 16	0.002 - 16	0.002 - 1
oxacillin	0.008 - 128		
penicillin	0.002 - 16	0.002 - 16	0.002 - 2
piperacillin-tazobactam ^{1,2} (4mg/L taz)	0.002 - 16 pip + 4 taz	0.002 - 16 pip + 4 taz	0.002 - 1 pip + 4 taz
teicoplanin	0.03 - 16	0.03 - 16	0.03 - 16
tetracycline	0.06 - 128	0.06 - 128	0.06 - 128
vancomycin	0.06 - 16	0.06 - 16	0.06 - 16

Gram-negative isolates

DRUG	Enterobacteriaceae mg/L	<i>Pseudomonas</i> mg/L
amoxicillin	0.25 - 256	
amoxicillin- clavulanate ³ (2:1)	0.12 - 64	
cefotaxime	0.008 - 16 (*256)	
cefoxitin	0.5 - 128	
ceftazidime	0.008 - 16 (*256)	0.03 - 16 (*256)
cefuroxime	0.12 - 128	
ciprofloxacin	0.008 - 16 (*256)	0.002 - 16 (*256)
gentamicin	0.12 - 16 (*256)	0.12 - 16 (*256)
imipenem ¹	0.008 - 16 (*256)	0.03 - 16 (*0.008, 256)
piperacillin- tazobactam ^{1,2}	0.015 - 256 (32 for Proteeae)	0.12 - 64 (*256)
tetracycline	0.12 - 128	
trimethoprim- sulphamethoxazole		

¹ Poor stability - plates containing these agents to be used on day of preparation.

² Test with tazobactam at 4 mg/L fixed concentration; reported concentrations refer to piperacillin.

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

(*) Extend range as shown in brackets if MIC is outside the initial range tested.

ST - *S. aureus*; CN - coagulase-negative staphylococci

7 Detection of Mechanisms of Resistance, and Related Issues of Testing

Extended-spectrum β -lactamases - ESBLs

All isolates of Enterobacteriaceae with ceftazidime or cefotaxime MICs on or above the susceptibility breakpoint will be tested with ceftazidime, cefotaxime and ceftazidime, each \pm 4 mg/L clavulanate. ESBL production is inferred if any of these three MICs is reduced \geq 8-fold by clavulanate. An exception is made for *K. oxytoca* highly resistant (commonly, MIC \geq 64) to piperacillin/tazobactam and cefuroxime but not to cefotaxime and ceftazidime (probable K1 enzyme hyperproducers), which can give false positive results in clavulanate synergy testing (with cefotaxime, ceftazidime or ceftazidime, but not ceftazidime): these are excluded from the count of ESBL producers.

The MIC recorded in the dataset will generally be that measured originally, except when the initial value is censored (e.g. >16 mg/L) and the subsequent value is an exact result compatible with the original (e.g. 256 mg/L), in which case the subsequent exact result will be recorded.

CTX-M enzymes

ESBL-positive Enterobacteriaceae with cefotaxime MICs \geq 4-fold higher than for ceftazidime, or cefotaxime MICs >256 mg/L, will be tested by multiplex PCR to detect and characterise *bla*_{CTX-M} genes.

mecA

Staphylococci are tested by PCR to detect the presence of the *mecA* gene (encoding PBP-2')

mupA

Staphylococci are tested by PCR to detect the presence of the *mupA* gene (conferring high-level mupirocin resistance).

Inconsistencies between initial and subsequent tests

On rare occasions, an isolate may show very different MICs in initial and subsequent tests. For example, a highly cefotaxime-resistant isolate may be found cefotaxime-susceptible when later re-tested to ascertain ESBL status. This could be the result of a plasmid loss, which may also affect susceptibility to other antimicrobials not included in the re-test panel. If such a loss is inferred, the isolate will be retained in the dataset with its originally determined MICs, and its ESBL status will be recorded as not confirmed.

8 Quality Assurance

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 year.

9 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 31st July each year.

The complete data for each testing year will include a listing to show, for each isolate, the background information (patient age etc.), MIC for each agent tested, and information from any additional tests (e.g. for ESBLs).

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 year, and it will be made available in suitable summary form for at least a year through a website. Each year, 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 or, in the absence of BSAC guidance, EUCAST breakpoints (www.escmid.org).

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 may be combined to contribute one quota of isolates. If a laboratory withdraws from the programme, it will be replaced if possible with a laboratory serving an area nearby.

The 25 centres currently contributing (2009) are:

Collecting Laboratory	Town/City	Country
William Harvey Hospital	Ashford	England
Birmingham City Hospital	Birmingham	England
Bristol Royal Infirmary	Bristol	England
West Suffolk Hospital	Bury St. Edmunds	England
Addenbrooke's Hospital	Cambridge	England
Countess of Chester Hospital	Chester	England
Coventry & Warwickshire Hospital	Coventry	England
Leicester Royal Infirmary	Leicester	England
Northwick Park Hospital	London	England
St Mary's Hospital	London	England
Wythenshawe Hospital	Manchester	England
Freeman Hospital	Newcastle	England
Northern General Hospital	Sheffield	England
Royal Shrewsbury Hospital	Shrewsbury	England
Southampton General Hospital	Southampton	England
Sunderland Royal Hospital	Sunderland	England
Treliske Hospital	Truro	England
Cork University Hospital	Cork	Ireland
Beaumont Hospital	Dublin	Ireland
Antrim Area Hospital	Antrim	N. Ireland
Belfast City Hospital	Belfast	N. Ireland
Southern General Hospital	Glasgow	Scotland
Raigmore Hospital	Inverness	Scotland
Ysbyty Gwynedd	Bangor	Wales
University Hospital of Wales	Cardiff	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 Bacteraemia Resistance Surveillance Programme has run since 2001, based on an original protocol dated 7 February 2001. The following differences from the current protocol have existed at different times.

Exclusion of isolates

De-duplication period Up till 2007, isolates taken within one week of a previous clinically significant bacteraemia were excluded; the period was extended to two weeks from 2008.

Selection of isolates

Serratia and other Gram-negative organisms *Serratia* was collected as a separate group from 2008 onwards. From 2001 to 2007, instead of *Serratia*, Gram-negative organisms other than *E. coli*, *Klebsiella*, *Enterobacter*, Proteaeae, and *Pseudomonas* were collected as a group (but *Salmonella typhi* and *Neisseria* spp. were excluded). This 'other Gram-negative' group comprised approximately 39% *Serratia*, 15% *Acinetobacter*, 15% *Stenotrophomonas*, and 12% *Citrobacter*, with the remainder from a large variety of genera including very occasional anaerobes. Most genera therefore had too few representatives for meaningful analysis.

Number of isolates

S. aureus and *E. coli* The number of isolates of *S. aureus* and *E. coli* was increased to 20 per collecting laboratory, 500 in total per year, from 2008 onwards. From 2001 to 2007, the collection target for all organism groups was 10 per lab, 250 in total per year.

Identification of isolates

E. coli and *P. aeruginosa* In 2001 and 2002, *E. coli* and *P. aeruginosa* were identified using API20E and API20NE strips respectively. Experience from these years showed that the simpler procedures now specified in the protocol would have little or no impact on the accuracy of identification of these species.

Coagulase-negative staphylococci From 2001-2005 inclusive, coagulase-negative staphylococci were identified using API-STAPH and PCR, allowing a species identification to be made in most cases.

Misidentified and over-quota isolates, and mixed cultures

The following approach to 'misidentified, duplicate and over-quota' isolates was applied up to and including 2006. A change was made from 2007, after which only isolates identified as Enterobacteriaceae by both the collecting and central laboratory could be moved between study groups (paragraph 1). At the same time, paragraph 3 was amended to allow use of isolates from mixed cultures only in cases of obvious mild contamination.

- 1 If a collecting laboratory submits an isolate of a defined collecting group (e.g. *E. coli*) under another name (e.g. as 'other Gram-negative'), the isolate will be included in the study in its proper group (e.g. *E. coli*) provided that this does not bring the total of isolates in that group from that laboratory over the quota of ten.
- 2 If a collecting laboratory submits more than the quota of ten isolates of a defined collecting group, excess isolates will be excluded starting with any that were submitted under other names and then by date of collection (latest first).
- 3 On the rare occasions when a single blood sample yields two isolates of a single species but with different susceptibility profiles, the more susceptible isolate will be excluded from the study.

Care settings

In 2001, care setting data was not collected for patients from the Accident and Emergency speciality. However, on investigation, these blood samples were overwhelmingly from patients awaiting formal admission to hospital, so the care setting was imputed as 'in-patient, <48 hours' for all of them.

Agents for testing and testing ranges

The continuity group has been adjusted since testing began in 2001, and the testing ranges have varied slightly over the first few years of the study, although the aim has always been to minimise the reporting of censored MICs.

Amoxicillin-clavulanate: In 2001 and 2002, Enterobacteriaceae were tested with a fixed concentration of clavulanate (2 mg/L) as well as the 2:1 ratio amoxicillin:clavulanate.

Cefotaxime: Full MIC testing of Enterobacteriaceae with cefotaxime began in 2003. In 2002 they were tested only with the then breakpoint concentration 1 mg/L, and were not tested at all in 2001.
Cefoxitin: Testing of staphylococci (at both 37°C and 30°C) was undertaken in 2004.
Clindamycin: Testing of anaerobes began in 2002
Fusidic acid: Testing of staphylococci began in 2004.
Imipenem: Staphylococci were tested from 2001 to 2002.
Metronidazole: Testing of anaerobes began in 2002.
Mupirocin: Testing of staphylococci began in 2007.
Oxacillin: Streptococci other than *S. pneumoniae* were tested from 2001 to 2004.
Rifampicin: Testing of staphylococci began in 2004.
Tetracycline: Testing of Gram-negative organisms began in 2002.
Trimethoprim-sulphamethoxazole: Testing of *S. maltophilia* began in 2004.
Piperacillin-tazobactam: Added to continuity group in 2009. (Tested 2001-2008 under sponsorship from Wyeth.)

Detection of Mechanisms of Resistance

ESBL testing

The testing regime for ESBL has evolved, as different enzymes became more prevalent and recommendations changed, especially over the early years of the study. Thus there are some potential minor discrepancies, particularly between ESBL rates recorded in 2001-2003 and later years.

In 2001, Enterobacteriaceae (except *Serratia*) resistant to ceftazidime were tested further for ESBL production (using ceftazidime and cefotaxime, each ± clavulanate). While this procedure detects TEM and SHV variants, it might leave CTX-M ESBLs undetected.

From 2002, cefotaxime was added to the screening process for Enterobacteriaceae. Isolates (except *Serratia*) resistant to either ceftazidime or cefotaxime were tested further (using ceftazidime, cefotaxime, and cefepime, each ± clavulanate) thus detecting TEM, SHV and CTX-M.

From 2003 ALL Enterobacteriaceae without exception (i.e. including *Serratia*) were screened for ESBL production by examination of their cefotaxime and ceftazidime MICs.

From 2001 to 2003, the official criterion for further testing was 'resistant to cefotaxime or ceftazidime' but occasional isolates on the susceptibility breakpoint with 'suspicious' antibiograms were also tested.

From 2005, the synergy testing used ceftazidime, cefotaxime, and cefpirome, each ± clavulanate (i.e. cefpirome replaced cefepime).

Summary of screening rules:

2001: *Serratia* not screened, no cefotaxime screen, isolates tested if ceftazidime-resistant or suspicious.

2002: *Serratia* not screened, isolates tested if ceftazidime-resistant, cefotaxime-resistant, or suspicious.

2003: All Enterobacteriaceae screened, isolates tested if ceftazidime-resistant, cefotaxime-resistant, or suspicious.

2004-2006: All Enterobacteriaceae screened, isolates tested if ceftazidime MIC ≥2 mg/L or cefotaxime MIC ≥1 mg/L (i.e. on or above the susceptibility breakpoints defined in BSAC method, versions 3-5, Jan 2004 - Jan 2006).

2007 onwards: All Enterobacteriaceae screened, isolates tested if ceftazidime MIC ≥1 mg/L or cefotaxime MIC ≥1 mg/L (i.e. on or above the susceptibility breakpoints defined in BSAC method, version 6, Jan 2007).

Summary of synergy testing methods:

2001: ceftazidime and cefotaxime, each ± clavulanate 4mg/L.

2002-4: ceftazidime, cefotaxime and cefepime, each ± clavulanate 4mg/L.

2005 onwards: ceftazidime, cefotaxime and cefepirome, each ± clavulanate 4mg/L.

CTX-M testing

The selection of isolates for CTX-M testing was not formally specified up to 2007.

mecA

mecA testing was introduced for *Staphylococcus aureus* in 2005 and for all staphylococci in 2006.

Staphylococci are considered to be 'methicillin-resistant' if they are phenotypically resistant to oxacillin by breakpoint or if they have the *mecA* gene.

mupA

mupA testing was introduced for staphylococci in 2006.

Inconsistencies between initial and subsequent tests

Up to and including 2006, those few isolates that gave persistently inconsistent results between initial and subsequent tests (for example, a highly cefotaxime-resistant isolate found persistently cefotaxime-

susceptible during ESBL synergy testing) were excluded from the database. The change to include these isolates was made from 2007 onwards.

Changes to Collecting Laboratories

Now	Previously
Sunderland Royal Hospital. From 2002.	Missing, except one isolate from Middlesbrough. 2001
Addenbrooke's Hospital, Cambridge. From 2002.	Chelmsford Public Health Laboratory. 2001
Victoria Hospital, Kirkcaldy From 2002.	Ninewells Hospital, Dundee. 2001
West Suffolk Hospital, Bury St. Edmunds. From 2002.	Norfolk and Norwich Hospital, Norfolk. 2001
Leicester Royal Infirmary. From 2003.	University Hospital, Nottingham. 2001-2002
Victoria Infirmary, Glasgow From 2007.	Glasgow Royal Infirmary 2001-2006
Southern General Hospital, Glasgow. From 2009	Victoria Infirmary, Glasgow 2007-2008.
Raigmore Hospital, Inverness From 2009	Victoria Hospital, Kirkcaldy 2002-2008
Antrim Area Hospital, Antrim From 2009	Altnagelvin Area Hospital, Londonderry 2001-2008
Northwick Park Hospital, London From 2009	University College Hospital, London 2001-2008

Sensitivity Testing of Isolates

The method summarised below was used for isolates collected up to and including 2007.

The description was much simplified from 2008 (version 2.5) onwards when the 'Other Gram-negative bacteria' group was replaced by *Serratia*, though the method itself was unchanged.

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

Culture Conditions - Gram Negative Organisms

Organisms	medium	supplements	spot size	atmosphere	temperature	incubation time
<i>E. coli</i> , <i>Klebsiella</i> , non-swarming Proteaeae, <i>Enterobacter</i> , <i>Pseudomonas</i>	Iso-sensitest agar	-	10 ⁴	air	35 - 37°C	18 - 20 hours
swarming Proteaeae e.g. <i>Proteus</i> spp.	Iso-sensitest agar	50 g/L PNPG	10 ⁴	air	35 - 37°C	18 - 20 hours
Other Gram-negative organisms: if not listed below, see description in JAC 2001 or BSAC website (www.bsac.org.uk) and use appropriate special methods.						
<i>Moraxella</i> but not with β-lactams ¹	Iso-sensitest agar	5% defibrinated horse blood	10 ⁴	air	35 - 37°C	18 - 20 hours
<i>Haemophilus</i>	Iso-sensitest agar	5% defibrinated horse blood + 20mg/L NAD	10 ⁴	air + 4-6% CO ₂	35 - 37°C	18 - 20 hours
<i>Neisseria</i>	Iso-sensitest agar	5% defibrinated horse blood	10 ⁴	air + 4-6% CO ₂	35 - 37°C	18 - 20 hours

Culture Conditions - Gram Positive Organisms

Staphylococci but not with methicillin, oxacillin, penicillin	Iso-sensitest agar	-	10 ⁴	air	35 - 37°C	18 - 20 hours
<i>Enterococcus</i> spp.	Iso-sensitest agar	-	10 ⁴	air	35 - 37°C	18 - 20 hours
<i>S. pneumoniae</i> & other α- & non-haemolytic streptococci	Iso-sensitest agar	5% defibrinated horse blood	10 ⁴	air + 4-6% CO ₂	35 - 37°C	18 - 20 hours
β-haemolytic streptococci	Iso-sensitest agar	5% defibrinated horse blood	10 ⁴	air	35 - 37°C	18 - 20 hours

Culture Conditions - Special Cases (Gram-negative and Gram-positive) Change from usual conditions for that species shown in bold.

organisms	special case	medium	supplements	spot size	atmosphere	temperature	incubation time
Staphylococci	methicillin oxacillin	Columbia agar	2% NaCl	10 ⁴	air	30°C	24 hours
Staphylococci	penicillin	Iso-sensitest agar	-	10⁶	air	35 - 37°C	18 - 20 hours
<i>Moraxella</i>	β-lactams	Iso-sensitest agar	5% defibrinated horse blood	10⁶	air	35 - 37°C	18 - 20 hours

¹The method specifies the increased inoculum for *Moraxella* with ampicillin and amoxicillin, but we take it to apply to all β-lactam antimicrobials.

MICs for anaerobic organisms and category 3 pathogens requiring special containment will be measured by E-test, with the culture conditions of the BSAC standardised method.