RESISTANCE SURVEILLANCE
PROGRAMME

BACTERAEMIA PROTOCOL 2019

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

Version 2.0 January 2019
Applies to the Isolate Collection Period from 1st January 2019 to 31st December 2019

BRITISH SOCIETY FOR ANTIMICROBIAL CHEMOTHERAPY

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1. SUMMARY

1.1 Study Title

BSAC Bacteraemia Resistance Surveillance Programme.

1.2 Initiator

The British Society for Antimicrobial Chemotherapy (BSAC), 53 Regent Place, Birmingham, B1 3NJ.

1.3 Funding

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

1.4 Objective

Determination of the antimicrobial susceptibility of currently circulating bacterial isolates from clinically significant bacteraemia.

1.5 Central Testing Laboratory

Public Health England (PHE), Colindale, London.

1.6 Geographical Scope

Twenty-five collecting centres have been, or will be, selected to give good geographical spread throughout the United Kingdom and Ireland. The number of centres may be reduced and the target number of isolates per centre increased in future if more centres merge and the populations they cover increases.

1.7 Selection and numbers of Isolates

Twelve groups of organisms (six Gram-negative, six Gram-positive) will be collected as bloodstream isolates recovered by participating laboratories, from patients with clinically significant bacteraemia, excluding repeat isolates within 14 days, which are assumed to be from the same episode of infection. The groups include those organisms that are considered to be the most common agents of bacteraemia.

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

Each centre will collect up to 20 consecutive isolates of *Staphylococcus aureus* and *Escherichia coli* and up to 8-12 consecutive isolates in each other organism group, giving a target total of 500 isolates of *S. aureus*, 500 *E. coli*, and 200-300 isolates in each other group.

1.8 Testing of Isolates

The isolates will be re-identified by the Central Testing Laboratory and tested using the BSAC agar dilution method for the determination of minimum inhibitory concentrations. Further tests will be used to identify selected mechanisms of resistance and to type selected groups of organisms.

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

Each centre will collect consecutive isolates of the organisms shown in Table 1 below, up to the number shown, and according to the inclusion/exclusion criteria.

Table 1. Organisms collected by the BSAC Bacteraemia Resistance Surveillance project.

Gram-positive bacteria	N	Gram-negative bacteria	N
Staphylococcus aureus	20	*Escherichia coli*	20
Coagulase-negative staphylococci	10	****Klebsiella* spp.**	**12**
Streptococcus pneumoniae	10	****Enterobacter* spp.**	**8**
other α- & non-haemolytic streptococci	10	Proteeae	10
β-haemolytic streptococci	10	*Serratia* spp.	10
Enterococcus spp.	10	*Pseudomonas* spp.	10

* Please note that changes to the total number of *Klebsiella* species and *Enterobacter* species requested have been made since the last version of the protocol. This is due to a change in taxonomy/nomenclature: *E. aerogenes* is now known as *K. aerogenes*.

2.3 Inclusion Criteria

1. Isolates from blood.
2. Patients with bacteraemia judged clinically significant by the responsible medical microbiologist
3. Collected between 1st January and 31st December of the relevant year.

2.4 Exclusion Criteria

1. Repeat isolates from the same infection episode i.e. isolates obtained within two weeks of a previous isolation of the same species from a clinically significant bacteraemia in the same patient. Note that isolates of different species collected from the same clinical episode are not excluded.

2.5 Discrepant Identifications, Over-Quota Isolates, and Mixed Cultures

1. In general, an isolate will be excluded if central and collecting laboratory identifications place it in different organism collection groups, and will remain eligible for inclusion (under its central laboratory identification) if both laboratories place it in the same collection group. Enterobacteriaceae are a special case: an isolate submitted and confirmed as Enterobacteriaceae will be eligible for inclusion in a different Enterobacteriaceae group (under its central laboratory identification), providing that this does not bring the total of isolates in that group from that collecting laboratory over the quota of 20 for *E. coli* and, and 8-12 for other groups. Replacements for excluded isolates will be sought, up to the quota, if time remains in the collecting season.
2. If a collecting laboratory submits more than its quota of a defined collection 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.

2.6 Storage of Isolates in Collecting Laboratories

Isolates may be stored frozen in suitable media at or below -70°C for up to 12 months or at temperatures up to -20°C for shorter periods compatible with very high rates of recovery of viable organisms (no more than 2 months for *S. pneumoniae*). Thawed isolates should be sub-cultured onto non-selective medium to give luxuriant growth after overnight incubation before being prepared for transport to the Central Testing Laboratory.

2.7 Transport

Collecting laboratories should send isolates to the Central Testing Laboratory on agar slopes or by any other suitable method, and in compliance with prevailing transport regulations.

3. ADDITIONAL DATA TO BE SUPPLIED BY COLLECTING LABORATORIES

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:
  - Hospital-onset (inpatient >48 hours from admission)
  - Community-onset (GP patient, outpatient and hospital inpatient ≤48 hours from admission)
- For hospital patients, the requesting speciality, from the following categories:
  - intensive care unit
  - not from an intensive care unit
- Identification of isolate by genus and species, if known

4. REMUNERATION

Participating laboratories are remunerated for the isolates that are submitted to the surveillance programme on a yearly basis. The number of isolates confirmed to be suitable for inclusion (i.e. correct identification, no patient duplicates) will be remunerated. Isolates that fail to grow on receipt at the central testing laboratory or do not fit the inclusion criteria will not be included in the final total received by any one laboratory (See sections 2.3-2.5).

5. IDENTIFICATION AND STORAGE OF ISOLATES

5.1 Receipt

On receipt at the Central Testing Laboratory, the isolates will be sub-cultured on appropriate non-selective medium and checked for purity. Following identification, isolates not conforming to the criteria of section 2 above will be discarded without testing.

5.2 Initial Identification

The isolates will be identified by appropriate methods as detailed below. 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.

Matrix-assisted laser desorption time-of-flight mass spectrometry (MALDI-ToF) will be used to identify the majority of isolates, with identification to species level in most cases.

The following groups are expected to be identified by MALDI-ToF:

- Staphylococci (in conjunction with chromogenic media)
- Enterococci
- β-haemolytic streptococci
- Enterobacteriaceae other than *E. coli*
- *Pseudomonas*
- α- and non-haemolytic streptococci (ID to species group level)

Lancefield group for β-haemolytic streptococci will be inferred from the species identification where possible, and determined by Lancefield typing otherwise (e.g. *S. dysgalactiae*).

The following groups will be identified by the methods listed below:

- *E. coli*: Pink on chromogenic media. MALDI-ToF may be used as an alternative method of identification for *E. coli*.
- *S. pneumoniae*: serotyping by the standard methods of the PHE RVPBRU (Public Health England Respiratory and Vaccine Preventable Bacteria Reference Unit, London), comprising either classical serotyping or prediction from genomic sequence data.

Coagulase tests may be used as an alternative identification method to MALDI-ToF for staphylococci.

Isolates that give doubtful or unusual results, or exhibit antibiograms that are anomalous for their species identification, will be re-identified by a second method, generally with API20E® or API20NE® strips (bioMérieux UK Ltd) for Gram-negative bacteria, PCR for enterococci and Lancefield typing for β-haemolytic streptococci.

5.3 Storage of isolates

Isolates will be stored at or below –70°C using blood glycerol broth or other agreed established methods that are known to give a high probability of long-term recovery of viable organisms.

6. SUSCEPTIBILITY TESTING OF ISOLATES

Minimum inhibitory concentrations will normally be measured by the BSAC agar dilution method[1] summarised in Table 2. Special conditions may apply for other antimicrobials not included in the continuity group, for example Ca^{2+}-supplemented isotonic medium for daptomycin.

As *Proteus* require anti-swarming measures and therefore cannot be tested on the same plates as other Enterobacteriaceae, the testing of *Proteus* may be delayed until a sufficient number have been collected to justify a special run of MIC tests.

Table 2. Summary of the conditions and media used to measure the minimum inhibitory concentration of bacteria collected.

Organism	Medium	Supplements	Spot size (CFU/spot)	Atmosphere	Temperature Duration of incubation
S. pneumoniae & other α- & non-haemolytic streptococci	Iso-Sensitest agar	5% defibrinated horse blood	10^4	air plus 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
Staphylococci (tests other than oxacillin)	Iso-Sensitest agar	None	10^4	air	35–37°C 18–20 hours
Staphylococci (oxacillin)	Columbia agar	2% NaCl	10^4	air	30°C 24 hours
Enterococcus spp.	Iso-Sensitest agar	None	10^4	air	35–37°C 18–20 hours
Enterobacteriaceae (excluding swarming species)	Iso-Sensitest agar	None	10^4	air	35–37°C 18–20 hours
Swarming Enterobacteriaceae e.g. *Proteus* spp.	Iso-Sensitest agar	50 mg/L PNPG	10^4	air	35–37°C 18–20 hours
Pseudomonas spp.	Iso-Sensitest agar	None	10^4	air	35–37°C 18–20 hours

Key: PNPG: p-nitrophenyl glycerine.

7. ANTIMICROBIAL AGENTS FOR TESTING - TESTING RANGES AND INTERPRETATION

The isolates will be tested against a range of antimicrobial agents. The tests and agents listed below form the 'continuity group' (meaning core agents that are tested, at BSAC's behest, every year irrespective of sponsors) and are intended to be studied for the full term of the programme. Additional agents may also be tested.

The concentration ranges tabulated below are the planned initial testing ranges (Tables 3-5). In some cases, extended ranges (shown in brackets) 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 and avoid off-scale values 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'.

7.1 Interpretation of MICs

7.1.1 Categorisation

If isolates are to be categorised as susceptible/intermediate/resistant, EUCAST breakpoints (www.eucast.org) will be used or, in the absence of EUCAST guidance, Clinical and Laboratory Standards Institute (CLSI) breakpoints (https://clsi.org).

7.1.2 Inconsistencies between Initial and Subsequent MIC Tests

On rare occasions, an isolate may show very different MICs in initial and subsequent susceptibility tests. For example, a highly cefotaxime-resistant isolate may be found to be 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 antimicrobial agents 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.

Table 3. Continuity agents tested - staphylococci and enterococci.

Antimicrobial Agent	Staphylococci (mg/L)	Enterococci (mg/L)
Ampicillin		0.03–128
Ciprofloxacin	0.03–128	
Clindamycin	0.03–128	
Clindamycin-erythromycin[1]	0.5 cli + 4 ery	
Erythromycin	0.03–128	
Fusidic acid	0.015–256	
Gentamicin	0.008–128	0.5–2048
Mupirocin	0.06–1024	
Oxacillin	0.03–128	
Rifampicin	0.004–2 (ST) 0.004-256 (CN)	
Streptomycin		8–1024
Teicoplanin	0.06–16	
Tetracycline	0.06–128	
Trimethoprim	0.06–128	
Vancomycin	0.25–16	0.12–128

[1] This is a breakpoint test to identify inducible resistance to clindamycin.

ST: *S. aureus*; CN: Coagulase negative staphylococci.

Table 4. Continuity agents tested - streptococci.

Antimicrobial Agent	*S. pneumoniae* (mg/L)	Other α- & non-haemolytic streptococci (mg/L)	β-haemolytic streptococci (mg/L)
Amoxicillin	0.004–16		
Cefotaxime	0.004–4	0.004–4	
Ciprofloxacin	0.25–128		
Clindamycin	0.015–128	0.015–128	0.008–128
Clindamycin-erythromycin[1]	0.5 cli + 4 ery	0.5 cli + 4 ery	0.5 cli + 4 ery
Erythromycin	0.015–128	0.015–128	0.015–128
Gentamicin		0.06–128	
Meropenem[2]	0.002–1		
Penicillin	0.002–16	0.002–16	0.002–2
Teicoplanin	0.03–16	0.03–16	0.03–16
Tetracycline	0.03–128	0.03–128	0.03–128
Vancomycin	0.06–16	0.06–16	0.06–16

[1] This is a breakpoint test to identify inducible resistance to clindamycin.

[2] Poor stability: plates containing these agents to be used on day of preparation.

Table 5. Continuity agents tested - Gram-negative isolates.

Antimicrobial Agent	Enterobacteriaceae (mg/L)	*Pseudomonas* (mg/L)
Amoxicillin	0.25–256	
Amoxicillin-clavulanate[1,2]	0.12–64	
Carbenicillin		32–256
Cefotaxime	0.008–16 (*256)	
Cefoxitin	0.5–128	
Ceftazidime	0.008–16 (*256)	0.03–16 (*256)
Ciprofloxacin	0.002–16 (*256)	0.002–16 (*256)
Colistin[3,4]	0.03–256	0.03–32
Gentamicin	0.12–16 (*256)	0.12–16 (*256)
Meropenem[2]	0.004 –2 (*256)	0.03–32
Piperacillin- tazobactam[2,5]	0.015–256 [*32 (P)]	0.12–64 (*256)
Tobramycin	0.12–16 (*256)	0.12–16 (*256)
Trimethoprim	0.03–16 (*256)	

[1] Test with clavulanate at 2 mg/L fixed concentration; reported concentrations refer to amoxicillin.

[2] Poor stability: plates containing these agents to be used on day of preparation.

[3] Excluding *Serratia* and Proteeae.

[4] Full range for colistin tested in first run as colistin resistance is suspected to be unstable.

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

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

(CN) coagulase-negative staphylococci; (P) Proteeae.

8. FURTHER TESTING - DETECTION OF MECHANISMS OF RESISTANCE AND ADDITIONAL TYPING

8.1 Planned Further Phenotypic Testing

8.1.1 Extended-spectrum β-lactamases (ESBLs), AmpC enzymes and K1 hyperproduction

All isolates of Enterobacteriaceae with ceftazidime or cefotaxime MICs on or above the susceptibility breakpoint (i.e. ≥1 mg/L), and isolates of *Klebsiella oxytoca* with piperacillin-tazobactam MICs ≥128 mg/L, will be tested further (by BSAC agar dilution or MIC gradient strip test):

- For ESBL activity - using the clavulanate synergy test with ceftazidime, cefotaxime and cefepime each ± 4 mg/L clavulanate. Cefpirome is an acceptable substitute for cefepime when cefepime is unavailable.
- For AmpC activity - using the cloxacillin synergy test with cefotaxime ± 100 mg/l cloxacillin for BSAC agar dilution or with cefotetan ± cloxacillin for MIC gradient strip test.
- In addition, isolates of *K. oxytoca* with piperacillin-tazobactam MICs ≥128 mg/L will be tested against aztreonam and cefuroxime for detection of K1 hyperproduction by interpretive reading2

Swarming Enterobacteriaceae e.g. *Proteus* spp. will normally be tested by MIC gradient strip test.

ESBL production is inferred if any (but generally all) of the three cephalosporin MICs are reduced ≥8-fold (i.e. by ≥3 doubling dilutions) by clavulanate. An exception is made for isolates of *K. oxytoca* considered to be K1 hyperproducers (see below) as these can give weak false positive results in clavulanate synergy testing with cefotaxime, cefepime or cefpirome, but not ceftazidime.

AmpC production is generally inferred if the cefotaxime MIC is reduced ≥4-fold (i.e. by ≥2 doubling dilutions) by cloxacillin, but interpretation may be modified by reference to the whole antibiogram to allow for unusual or multiple mechanisms of resistance. For example, in general, isolates with copious AmpC are more susceptible to cefepime/cefpirome than to ceftazidime and cefotaxime, but derepressed AmpC in *Serratia* has little effect on ceftazidime.

K. oxytoca that are highly resistant to piperacillin-tazobactam (≥128 mg/L), resistant to cefuroxime, no more than borderline resistant (i.e. with MICs around the breakpoint, either slightly above or slightly below) to cefotaxime and susceptible to ceftazidime will normally be interpreted as K1 hyperproducers and not as ESBL producers.

The Central Testing Laboratory will supply all the individual MICs obtained in the synergy tests for ESBL/AmpC detection to the BSAC in addition to the data for individual cephalosporins in the main dataset. The MIC recorded in the main 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.

8.2 Planned Additional Genotypic Testing

8.2.1 CTX-M ESBLs

Isolates inferred to have ESBLs (based on cephalosporin-clavulanate synergy) will be subjected to type-specific PCR for $bla_{CTX\text{-}M}$[3] if the isolate is resistant to either or both of cefotaxime and ceftazidime and if the cefotaxime MIC is higher than the ceftazidime MIC.

8.2.2 AmpC

E. coli, *Klebsiella* and *P. mirabilis* inferred to have AmpC-mediated resistance will be subjected to PCR for plasmid-mediated AmpC[4].

8.2.3 Carbapenemases

For Enterobacteriaceae (except Proteeae), a 'Phenotypic Testing' run, comprising ceftazidime, cefotaxime and cefepime each +/- 4 mg/L clavulanate, cefotaxime + 100 mg/L cloxacillin, imipenem +/- EDTA, temocillin, aztreonam and cefuroxime is completed; resistance mechanisms are inferred from these results, plus those of the main antimicrobial panel. Proteeae are tested against cefepime each +/- 4 mg/L clavulanate, cefotaxime + 100 mg/l cloxacillin, by gradient strip.

For carbapenem-resistant isolates the following possibilities are considered:

- Possible metallo-beta-lactamase (MBL): if the isolate is resistant to a cephalosporin with no synergy with clavulanate or cloxacillin, and is resistant to carbapenems with imipenem/EDTA synergy ≥8 fold.
- Possible Class A carbapenemase (e.g. KPC): if the isolate is resistant to carbapenems, and there is no imipenem/EDTA synergy, however, the isolates does show imipenem-relebactam synergy and ceftazidime-avibactam synergy.
- Possible OXA-48-like: if the isolate is resistant to at least one carbapenem, there is no imipenem/EDTA synergy, and the temocillin MIC is ≥128mg/L.

The AusDiagnostics RT-PCR system (which detects VIM, IMP, SME, OXA-48-like, KPC, NDM, SIM, FRI, IMI, SPM, GES)[5] is used to detect the presence of carbapenemase genes in isolates that are flagged according to the above criteria.

Pseudomonas with high-level resistance (≥4mg/L) to ceftolozane-tazobactam will be examined for corresponding carbapenemase genes by molecular methods.

In case of outbreaks, or if more than 25 isolates are candidates for carbapenemase testing, selection criteria may be developed and applied.

8.2.4 *mec*A

All staphylococci will be tested to detect the presence of the *mec*A gene (encoding PBP-2') by an in-house multiplex PCR.[6]

8.2.5 *mup*A

All staphylococci will be tested to detect the presence of the *mup*A gene (conferring high-level mupirocin resistance) by an in-house multiplex PCR.[7]

8.3 Additional investigations of exceptional resistances and resistance clusters

8.3.1 Unusual Resistances

Exceptional resistances of public health importance will be investigated. In general, these are those that would have been investigated on the reference service of the PHE Antimicrobial Resistance and Healthcare Associated Infections Unit (AMRHAI) had the same isolate been submitted to AMRHAI by the collecting laboratory, for example:

- linezolid-resistant Gram-positive bacteria (examined for G2576T, other rRNA mutations, or *cfr* or optrA)
- oxacillin-resistant *S. aureus* lacking *mec*A (examined for *mec*C)
- any penicillin-resistant β-haemolytic streptococci

8.3.2 Clusters

Typing will be undertaken on clusters of exceptionally resistant isolates. In general, these are those that would have been investigated by AMRHAI under its own remit for public health purposes had they been detected in other circumstances e.g. major clusters of carbapenemase-producers at a single hospital, or where unexpected phenotypes (e.g. colistin-resistant *Enterobacter*) are found to be geographically disseminated.

9. QUALITY ASSURANCE

9.1 Internal Quality Control

The Central Testing Laboratory will supply data from internal controls. The strain types used are listed in Table 6, including MICs and identification of test runs at the same time as the data from collected isolates.

Table 6. Strain types used for quality control purposes.

Enterobacteriaceae	
NCTC 10418	*E. coli*
NCTC 11954	*E. coli*
NCTC 11560	*E. coli*
ATCC 25922	*E. coli*
Pseudomonas	
ATCC 29213	*S. aureus*
ATCC 27853	*P. aeruginosa*
NCTC 10662	*P. aeruginosa*
NCTC 11560	*E. coli*
Enterococci	
NCTC 6571	*S. aureus*
ATCC 29212	*E. faecalis*
ATCC 29213	*S. aureus*
Staphylococci	
NCTC 11561	*S. aureus*
ATCC 29213	*S. aureus*
ATCC 12228	*S. epidermidis*
NCTC 6571	*S. aureus*
ATCC 43300	*S. aureus*
ATCC 29212	*E. faecalis*
ATCC 25923	*S. aureus*
Streptococci	
ATCC 49619	*S. pneumoniae*
CTL 4894	*E. faecalis*
CTL 6357	*S. pyogenes*
NCTC 11561	*S. aureus*
ATCC 29213	*S. aureus*
Synergy testing	
NCTC 10418	*E. coli*
NCTC 11560	*E. coli*
ATCC 25922	*E. coli*
ATCC 35218	*E. coli*
ATCC 27853	*P. aeruginosa*
PS10586	*P. aeruginosa*
PS2297	*P. aeruginosa*
ATCC 700603	*K. pneumoniae*

Key: Strains beginning with CTL or PS are internal control strains; ATCC, American Type Culture Collection; NCTC, National Collection of Type Cultures

10. DATA HANDLING

There will be suitable safeguards to ensure that data is entered into the study records accurately, maintained securely, and disseminated in encrypted form only to authorised recipients.

The complete and final data will be supplied to the BSAC by the Central Testing Laboratory by 31st July following the isolate collection period.

The complete data for each isolate will include a listing to show demographic information, MIC of each agent tested, information about the testing run number for each isolate (so that study results can be matched to control results and any runs producing unusual results can be identified) and information from any additional tests (e.g. MICs from ESBL synergy tests, results from molecular tests, interpretive readings of phenotypic tests, and all other data produced or received about the study isolates).

All other data related to the study such as the total number of bacteraemia isolates reported to PHE surveillance schemes by each laboratory and results for quality control isolates will be supplied at the same time as the data on study isolates.

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. Each year, the BSAC will circulate the data line-listed by isolate to all full sponsors for that year and in suitable summary form through the BSAC Resistance Surveillance Programme website (http://www.bsacsurv.org/).

11. COLLECTING LABORATORIES

A total of 25 centres will be contributing for the 2019 programme. Each participating laboratory is anonymised by the assignment of a unique individual code.

The set of collecting laboratories is selected to give good geographical coverage of the United Kingdom and Ireland, with a range of catchments (e.g. urban/rural, teaching/non-teaching hospitals). The same set should contribute to both BSAC Resistance Surveillance Programmes (Bacteraemia and Respiratory).

The Central Testing Laboratory is responsible for the recruitment of replacement collecting laboratories, subject to approval by the BSAC. If a laboratory withdraws from the programme, it will be replaced using the following criteria as far as possible:

- in the same geographical area as the laboratory it is replacing, or in an area that is under-represented;
- of the same type as the laboratory it is replacing (e.g. district general hospital vs tertiary referral centre), or of a type that is under-represented;
- having the staff commitment and organisational capacity to contribute isolates reliably according to the protocol.

12. PROTOCOL AMENDMENTS

12.1 Future Amendments

Amendments to this protocol can be made by agreement of the BSAC Standing Committee on Resistance Surveillance.

From January 2018, all amendments to this document will be recorded in the Amendment Table at the end of the document.

12.2 Historical Amendments

Historical amendments (from inception of the programme until December 2017) are recorded in Appendix 1.

13. FURTHER INFORMATION

Further information about the BSAC Resistance Surveillance Programme can be found on the associated website: http://www.bsacsurv.org/ or by contacting the BSAC Resistance Surveillance Programme Co-ordinator by telephone (0121 236 1988) or by email (rs@bsac.org.uk).

14. REFERENCES

1. Andrews JM. Determination of minimum inhibitory concentrations. *J Antimicrob Chemother* 2001; **48 Suppl 1**: 5-16.
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4. Perez-Perez FJ, Hanson ND. Detection of plasmid-mediated AmpC beta-lactamase genes in clinical isolates by using multiplex PCR. *J Clin Microbiol* 2002; **40**: 2153-62.
5. Meunier D, Woodford N, Hopkins KL. Evaluation of the AusDiagnostics MT CRE EU assay for the detection of carbapenemase genes and transferable colistin resistance determinants mcr-1/-2 in MDR Gram-negative bacteria. *J Antimicrob Chemother* 2018; 10.1093/jac/dky347.
6. Bignardi GE, Woodford N, Chapman A *et al*. Detection of the *mec*A gene and phenotypic detection of resistance in *Staphylococcus aureus* isolates with borderline or low-level methicillin resistance. *J Antimicrob Chemother* 1996; **37**: 53-63.
7. Woodford N, Watson AP, Patel S *et al*. Heterogeneous location of the *mup*A high-level mupirocin resistance gene in *Staphylococcus aureus*. *J Med Microbiol* 1998; **47**: 829-35.

AMENDMENT TABLE

This table of amendments is used to record information about changes that have been made to this document. A new version of the document will be given when major changes (multiple, large amendments) have been made, whereas an issue number will be given following minor changes (a single change or formatting/editorial changes).

Any suggestions for amendments to this document should be directed to: BSAC Resistance Surveillance Programme Co-ordinator, Email: RS@bsac.org.uk, Telephone: 0121 236 1988.

Date of change/Issue of Amendment	Section involved	Summary of changes	Author	New version number
10th December 2018	2.2	The number of *Enterobacter* spp. have been reduced from 10 to 8 and the number of *Klebsiella* spp. have been increased from 10 to 12. This is due to a change in taxonomy/nomenclature: *E. aerogenes* is now known as *K. aerogenes*.	CH	2
	8.2.3	Updated methods of carbapenemase detection.	CH	2
12th October 2018	All	Correction of grammatical errors and typos.	CH	1.3
	6	Removal of the following sentence: Future BSAC amendments to the original descriptions may be incorporated.	CH	1.3
	9.2	External Quality Assessment (EQA). Isolates of known antimicrobial susceptibility will be supplied to the Central Testing Laboratory by an external laboratory for testing by the methods of this programme each year. Results from these tests will be supplied at the same time as the data from collected isolates. As agreed by the RSP SC 31/10/17.	CH	1.3
	Amends	Amendment table moved from the beginning of the document to the end of the document.	CH	1.3
13th Sept 2017	1.2	Address added	CH	1.2
	1.7	Additional definition added	CH	1.2
	3	Removal of paragraph	CH	1.2
	4	Remuneration details added	CH	1.2
	5.2	Methods to identify *S. pneumoniae* changed Information added about α- non-haemolytic streptococci	CH	1.2
	5.3	Removal of further identification section	CH	1.2
	6	Additional paragraph added about Proteeae	CH	1.2
	7	Additional definition added	CH	1.2
	7.1.1	Paragraph updated	CH	1.2
	8.1	Definition of borderline resistance added	CH	1.2
	8.2	References added	CH	1.2
	8.2.3	Method changed from microarray to PCR	CH	1.2
	13	Further information sections added	CH	1.2
	14	References section added	CH	1.2

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APPENDIX 1. RECORD OF AMENDMENTS TO THE PROTOCOL

Past Differences

The BSAC Bacteraemia Resistance Surveillance Programme has run since 2001, based on an original protocol dated 7 February 2001 with 25 collecting laboratories. The last protocol describing surveillance with 25 collecting laboratories was version 2.7, 5th February 2010; it records amendments made between the 2001 and 2009 isolate collections.

From 2010, the surveillance was extended with the intention of collecting isolates from 40 clinical laboratories. Version 3.0 was the first protocol describing the programme with the extended collecting network. Later published versions were 3.2 and 3.3 (for 2011), 3.4 (for 2012) and 3.5 (for 2013); 3.1 was an unpublished draft. Version 3.5 records amendments made between the 2010 and 2013 collection periods.

Version 4.0 was the first protocol describing the programme following the consolidation of Central Testing Laboratory functions for both the Bacteraemia and Respiratory Resistance Surveillance Programmes at Public Health England, Colindale, and applies to the 2014 isolate collection period.

Changes for version 4.0, 19th August 2013 (compared with version 3.5): reprinted 8th Oct 2013

- Added accident & emergency (collected since about 2002) and respiratory medicine as new categories of hospital requesting specialities
- Clarified the testing conditions for ESBL and AmpC testing:
  - Use of cefepime in place of cefpirome for ESBL testing
  - Use of cefotaxime/cloxacillin combination for AmpC detection (has been in practice since 2003) except when Etests are used, then use of cefotetan/cloxacillin combination instead (also in practice since 2003)
  - Use of Etests for AmpC and ESBL testing of Proteeae (has been in practice since 2002)
- Added carbapenemase testing (existed earlier in practice, but was not specified in protocol).
- Harmonised the testing ranges for the core antimicrobial agents with those used in the Respiratory Resistance Surveillance Programme.
- Clarified when MALDI-ToF is used as identification method.
- Updated the list of collecting laboratories.

Changes for version 4.1, 29th May 2014 (compared with 4.0); still applies to 2013 collection period

- Amoxicillin-clavulanate to be tested with fixed 2 mg/L concentration of clavulanate, no longer 2:1 ratio.
- Further small amendments to testing ranges.
- Further update to collecting laboratories.

Changes for Bacteraemia Protocol 2014 v1 (19th August 2014) (compared with version 4.1)

- Document name changed to include the isolate collection period; version numbers will restart at 1 for each new period
- Typographical corrections: CFU/ml (not /spot); community-onset *S. pneumoniae* (not *S. aureus*)
- Imipenem dropped for all previously-tested organisms and replaced by meropenem for *Enterobacteriaceae*, *Pseudomonas*, *Acinetobacter*.
- Cefuroxime dropped for Enterobacteriaceae.
- Tobramycin added for Enterobacteriaceae, *Pseudomonas*, *Acinetobacter.*
- Carbenicillin added for *Pseudomonas* (with a view to a more targeted regime for future years).
- Altered provision for further β-lactamase testing in *Klebsiella oxytoca*. Isolates with piperacillin-tazobactam MIC ≥128 mg/L will now undergo further tests irrespective of MICs for ceftazidime and cefotaxime, and these tests will include measurement of aztreonam and cefuroxime MICs in order to better detect K1 hyper-producers.
- Piperacillin-tazobactam dropped for enterococci and all streptococci.

Changes for Bacteraemia Protocol 2015 v1.0 (14th April 2015) (compared with Protocol 2014 v1)

- New category for focus of infection “bone and joint”.
- Trimethoprim added for Enterobacteriaceae.
- Updated list of collecting laboratories.
- Working Party replaced with Standing Committee.

Changes for Bacteraemia Protocol 2015 v1.1 (4th June 2015) (compared with Protocol 2015 v1.0)

- Added list of internal control organisms in the methods section.
- Reduced testing range for carbenicillin.

Changes for Bacteraemia Protocol 2015 v1.1 (4th June 2015) (compared with Protocol 2015 v1.0)

- Increased the number of isolates collected per lab from 7 to 10 and 14 to 28
- Reduced the number of collecting laboratories from 40 to 25
- Dropped some of the data requested from collecting laboratories (laboratories’ own susceptibility data and origin of infection data)
- Simplified some of the data requested from collecting laboratories (speciality and care setting)
- Removed ciprofloxacin, erythromycin, penicillin and tetracycline for Enterococci, minocycline for Staphylococci, gentamicin and oxacillin for *S. pneumoniae*, amoxicillin and ciprofloxacin for other α- & non-haemolytic streptococci, amoxicillin, cefotaxime, ciprofloxacin and gentamicin for β-haemolytic streptococci, and tetracycline for Enterobacteriaceae
- Added streptomycin for Enterococci (range to be agreed at a later date)
- Stopped sending data systematically to every collecting laboratory