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TITLE:

Multi-centre study of the *in vitro* activity of ceftolozane/tazobactam and other commonly used antibiotics against *Pseudomonas aeruginosa* isolates from patients in the United Kingdom

(INVICTUS study)

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List of Abbreviations

BSAC	British Society for Antimicrobial Chemotherapy
CF	Cystic Fibrosis
ESBLs	Extended Spectrum ß-Lactamases
EUCAST	European Committee of Antimicrobial Susceptibility Testing
IAI	Intra-Abdominal Infection
ITU	Intensive Treatment Unit
MALDI-ToF MS	Matrix-Assisted Laser Desorption/ Ionization Time of Flight Mass Spectrometry
MDR	Multi Drug Resistant
MIC	Minimum Inhibitory Concentration
PACTS	Program to Assess Ceftolozane/Tazobactam Susceptibility
UTI	Urinary Tract Infection
XDR	Extremely Drug Resistant

Protocol Summary

Title	Multi-centre study of the *in vitro* activity of ceftolozane/tazobactam and other commonly used antibiotics against *Pseudomonas aeruginosa* isolates from patients in the United Kingdom
Chief Investigator	Dr Timonthy Planche
Rationale	*In vitro* studies conducted in Europe (eg. PACTS) and in the UK (BSAC) have demonstrated ceftolozane/tazobactam to be the most active beta-lactam antibiotic and second only to colistin against *P. aeruginosa*. Following the recent introduction of disc susceptibility testing methodology (a first-line test) it is important to ensure that testing is performed both accurately and consistently across centres in the UK and to provide centres with the opportunity to generate local susceptibility data to guide appropriate antimicrobial treatment for *P. aeruginosa*.
Primary Objective(s)	To evaluate the *in vitro* activity of ceftolozane/tazobactam and other commonly used antipseudomonal antibiotics against geographically spread *P. aeruginosa* isolates in the UK using disc testing.
Study Design	Multi-centre *in vitro* study to assess the susceptibility of *P. aeruginosa* to ceftolozane/tazobactam and other commonly used antibiotics using disc susceptibility testing methodology. Clinically significant consecutive isolates of *P. aeruginosa* starting from the most contemporaneous working backwards to be tested against ceftolozane/tazobactam and commonly used antibiotics from up to 25 centres in the UK with St. Georges Hospital, London as the designated central laboratory.
Study Population	Consecutive clinically significant isolates of *P. aeruginosa*
Study Duration	Seven months
Exposure and Outcome	*In vitro* activity of ceftolozane/tazobactam and other commonly used antibiotics against *P. aeruginosa* in the UK. Local susceptibility profile of *P. aeruginosa* isolates to ceftolozane/tazobactam. Evaluation of the EUCAST disc susceptibility testing method.
Statistical Methods	Descriptive analysis
Sample Size and Power Calculations	Up to 2500 clinically significant isolates of *P. aeruginosa*
Limitations	Potential for selection bias; inter-laboratory variability of sample type and results generated.

1 Background and Rationale

1.1 Background

Pseudomonas aeruginosa is an important causative pathogen in healthcare associated infections, including nosocomial pneumonia, urinary tract and bloodstream infections. In a point prevalence study conducted in ITU's in Western Europe, *Pseudomonas aeruginosa* was one of the most commonly reported pathogens, constituting 29% of all Gram-negative isolates and was present in 17% of all positive cultures [1]. In the ECDPC 2015 Report , *Pseudomonas aeruginosa* was identified as the leading cause of pneumonia (17%), the fourth most common isolated pathogen in urinary tract infections (14%), and responsible for 8% of bloodstream infections. *Pseudomonas aeruginosa* is also an important airways colonist in cystic fibrosis (CF) and chronic respiratory infection and is the main cause of morbidity and mortality in CF patients. [2,3] According to the UK Cystic Fibrosis Registry Annual Data Report 2016, there continues to be an increase in the total number of people in the UK living with CF (a total of nearly ten thousand and five hundred patients registered on the database). CF patients can present with both chronic and intermittent *P. aeruginosa* infections.[4]

Due to the low permeability of its outer-membrane, the constitutive expression of various efflux pumps and the production of antibiotic-inactivating enzymes, *Pseudomonas aeruginosa* is intrinsically resistant to several antibiotics. Useful activity against this pathogen is seen only among α-carboxy- and amino-penicillins, third- and fourth-generation cephalospoins, monobactams, carbapenems, aminoglycosides and fluoroquinolones. *Pseudomonas aeruginosa* also has a remarkable capacity to develop or acquire new mechanisms of resistance to antibiotics and resistance to all of these drug classes can arise by various chromosomal mutations resulting in upregulation of efflux pumps, downregulation of outer membrane porins or hyperproduction of chromosomal AmpC β-lactamase. [5] Resistance to β-lactams, aminoglycosides and quinolones can also arise by the acquisition of resistance determinants on mobile elements such as plasmids, transposons and integrons, with particular concerns centred around the acquisition of carbapenemases, with the Class B β-lactamases (metallo-betalactamases), the most frequently identified carbapenemases in *P. aeruginosa* isolates sent to the Reference Laboratory at Public Health England, Colindale, London [6]. Infections caused by resistant strains are a matter of concern in many hospitals worldwide, since they have been associated with higher costs and worse outcomes (eg, increased morbidity, mortality, length of hospital stay, and chronic care, higher rate of secondary bacteraemia) [5,7]. Treating such patients appropriately is important to ensure positive outcomes.Although repeated antibiotic treatments are associated with selection of resistance, which is more frequent in *P. aeruginosa* from CF than among *P. aeruginosa* from acute infections, antimicrobial resistance in CF is indeed a multifactorial problem. [2,3]

Ceftolozane, an oxyimino-cephalosporin, in combination with tazobactam, is licensed for the treatment of complicated intra-abdominal infections, complicated urinary tract infections and acute pyelonephritis. The addition of tazobactam to ceftolozane extends the activity to cover many ESBL-producing Enterobacteriaceae. [8]. Early *in vitro* studies have demonstrated ceftolozane to be notably active against *P. aeruginosa*, with MICs lower than ceftazidime, previously accepted as the most active cephalosporin against this species, in susceptible *P. aeruginosa* strains [6,9]. This activity of ceftolozane, which is independent of tazobactam, is retained for many strains with derepressed AmpC or up-regulated efflux, which are the major routes to resistance to established penicillins and cephalosporins in the species.[6] MICs of ceftolozane ranged from 0.5 to 2mg/L for isolates with upregulated efflux and ceftolozane retained good activity, with MICs≤4mg/L against mutants totally derepressed for AmpC. Full activity was also retained against OprD mutants resistant to imipenem.[9]

This activity has also been confirmed in large multi-centre studies in Europe, including Turkey and Israel, (Program to Assess Ceftolozane/Tazobactam Susceptibility- PACTS)[10–12] and the UK and Ireland (British Society for Antimicrobial Chemotherapy- BSAC- Resistance Surveillance Project)[13], with ceftolozane/tazobactam being the most active beta-lactam antibiotic and second only to colistin against *P. aeruginosa*.

Data from PACTS showed that 90.2% *P. aeruginosa* isolates recovered from hospitalised patients with a diagnosis of intra-abdominal infection were susceptible to ceftolozane/tazobactam. Amongst the same group of isolates, ceftolozane/tazobactam demonstrated superior *in vitro* activity to that of meropenem, piperacillin/tazobactam, ceftazidime, cefepime and ciprofloxacin.[10] A different study showed that 90.2% *P. aeruginosa* isolates recovered from patients hospitalised in intensive care units were susceptible to ceftolozane/tazobactam by EUCAST criteria. Susceptibility percentages were also high in MDR and XDR strains, being 75.1% and 52.3% respectively in this group of isolates[11]. Similarly, ceftolozane/tazobactam was active against 71.2% carbapenem-non-susceptible *P. aeruginosa* isolates from the PACTS study.[12]

Susceptibility data from the BSAC Resistance Surveillance Project in the UK shows ceftolozane/tazobactam as a potent antipseudomonal antibiotic *in vitro* with higher susceptibility rates than other beta-lactam/beta-lactamase inhibitor combinations, carbapenems and fluoroquinolones. Susceptibility rates have been consistently high over the five years analysed (2011-2015), with 99.5%, 99.6%, 100%, 100% and 99.6% bacteraemia isolates susceptible to this antibiotic. From respiratory isolates, susceptibility rates to ceftolozane/tazobactam were ≥90% for the 2010-2014 periods, second only to colistin. The recently published data from 2016 has confirmed the high susceptibility rates for ceftolozane/tazobactam seen over the previous years with a slight variation between bacteraemia (99.1% susceptible) and respiratory (98.1% susceptible) isolates. Although susceptibility rates for other commonly used antibiotics for the treatment of pseudomonal infections were also high, ceftolozane/tazobactam remained more active *in vitro* than other beta-lactam, beta-lactam/beta-lactamase inhibitor combinations, carbapenems, aminoglycosides and fluoroquinolones. A greater difference was observed between the two sets of isolates for these antibiotics, with lower susceptibility percentages in the respiratory programme (ceftazidime 92.3%, piperacillin/tazobactam 85%, meropenem 81.2%, imipenem 82.6%, amikacin 95.2%, gentamicin 95.2%, tobramycin 95.7% and ciprofloxacin 81.6%) than the bacteraemia one (ceftazidime 98.2%, piperacillin/tazobactam 95.4%, meropenem 91.2%, imipenem 91.2%, amikacin 99.5%, gentamicin 98.6%, tobramycin 98.2% and ciprofloxacin 89.4%). This on-going programme evaluates the antimicrobial susceptibility of currently circulating bacterial isolates from clinically significant infections and provides a random and geographically diverse sample from the UK and Ireland with more than twenty centres contributing to the programme every year.[13]

Antimicrobial susceptibility testing is performed routinely by the clinical microbiology laboratory to confirm susceptibility to chosen empirical antimicrobial agents, to identify alternative therapeutic options and to detect resistance in individual bacterial isolates. Automated or semi-automated methods such as broth microdilution or commercially marketed devices can provide results in a shorter period of time but are more expensive than manual tests. In addition, the drug selection is limited to those antimicrobials in standard commercial panels which do not normally include new antibiotics. On the other hand, although generally more labor intensive, manual methods provide flexibility and possible cost savings.[14] Disc susceptibility testing is a well-established, easy to performed and inexpensive methodology that is increasingly being adopted across UK laboratories as a first-line test. It is versatile in the range of antimicrobial agents that can be tested and requires no special equipment. [15]

1.2 Rationale

Infections caused by *P. aeruginosa,* particularly MDR *P. aeruginosa,* are a matter of concern in many hospitals worldwide since they can be associated with severe outcomes including increased mortality, hospital stay, and requirement for procedures.[16,17]

P. aeruginosa is the likely major target for ceftolozane/tazobactam; however, this species was poorly represented in the licensing trials. Although there are an increasing number of publications regarding the *in vitro* susceptibility of *P. aeruginosa* to ceftolozane/tazobactam, local data is still needed to guide appropriate antimicrobial treatment. In addition, knowing and understanding local sensitivity patterns of *P. aeruginosa* isolates can help to identify resistance trends and to plan treatment strategies for patients and testing protocols for laboratories.

Disc susceptibility testing offers clear advantages over currently available antimicrobial susceptibility testing methods for ceftolozane/tazobactam such as MIC gradient strips. It is inexpensive, and as a result of budget limitations in UK clinical laboratories, it is the preferred and, in many cases, the sole antimicrobial susceptibility testing method in an increasing number of sites in the UK. Following the recent publication of zone diameter breakpoints by EUCAST[18], it is important to ensure that this methodology is appropriately implemented in UK laboratories.

2 Objectives and Hypotheses

2.1 Primary Objective & Hypothesis

- To evaluate the *in vitro* activity of ceftolozane/tazobactam and other commonly used antipseudomonal antibiotics against geographically spread *P. aeruginosa* isolates in the UK.

2.2 Secondary Objectives & Hypotheses

- To provide centres with the opportunity to generate local susceptibility data that will guide appropriate antimicrobial treatment for *P. aeruginosa.*
- To assess the inter-laboratory reproducibility of results using the EUCAST disc susceptibility methodology.

3 Methodology

3.1 Summary of Study Design

This is a multi-centre, *in vitro* study to assess the susceptibility of clinically significant *P.aeruginosa* isolates against ceftolozane/tazobactam and other commonly used antipseudomonal antibiotics using disc susceptibility testing.

Re-identification of isolates, preferably by MALDI-ToF MS, and susceptibility testing following EUCAST methodology will be performed at the participating laboratories. St. Georges Hospital, London will act as the designated Central Testing Laboratory where isolates are to be sent for quality control activities and storage.

3.2 Centres

Similar to other well-stablished national susceptibility programmes, (BSAC Bacteraemia and Respiratory Resistance Surveillance Projects), the study will include up to twenty five centres to give good geographical spread throughout the United Kingdom. The majority of the centres will be tertiary or teaching hospitals, centres with large ITUs and CF units where pseudomonal infections are more likely to occur. The number of centres may be reduced and the target number of isolates per centre increased depending on the ability of the suggested centres to meet the inclusion criteria.

All study analysis (re-identification of *P. aeruginosa* isolates and antimicrobial susceptibility testing using the disc diffusion method) and data collection will be performed locally at each participant centre. St. Georges Hospital, London will act as the designated Central Testing Laboratory where all study isolates will be storage. Additionally, the Central Testing Laboratory will carry out quality control assays in a selected number of isolates per centre.

Participant centres

Centre	City	Contact
St. Georges Hospital	London	Dr Timothy Planche
Royal Harefield and Brompton	London	Dr Anne Hall
The Royal London	London	Dr Jonathan Lambourne
Imperial College London	London	Dr Hugo Donaldson
Lewisham University Hospital	London	Dr Tacim Kardag
University College Hospital	London	Prof Peter Wilson
Broomfield Hospital	Broomfield	Dr Wael Elamin
Epson and St Helier University Hospital	Epsom	Dr John Clark
Basildon University Hospital	Basildon	Dr Mustafa Awad-El-Kariem
Queen Alexandra Hospital	Cosham	Dr Andrew Flatt
Royal Devon and Exeter Hospital	Exeter	Dr Marina Morgan
Southmead Hospital Bristol	Bristol	Dr Karen Bowker
John Radcliffe Hosptial	Oxford	Dr Sarah Oakley
Heartlands Hospital	Birmingham	Dr Abid Hussain
Leicester Royal Infirmary	Leicester	Dr David Jenkins
Queen's Medical Centre	Nottingham	Dr Mathew Diggle
Royal Liverpool University Hospital	Liverpool	Dr Pankaj Lal
Pennine Acute Hosptial	Oldham	Dr Michael Pryzbylo
University Hospital South Manchester	Manchester	Dr Stephanie Thomas
Leeds General Infirmary	Leeds	Prof John Sandoe
Freeman Hospital	Newcastle	Prof John Perry
University Hospital of Wales	Cardiff	Dr Mandy Wootton
Belfast City Hospital	Belfast	Prof John Moore
Aberdeen Royal Infirmary	Aberdeen	Dr Noha El Sakka
Queen Elizabeth Hospital	Glasgow	Prof Alistair Leanord

3.3 Isolates

Up to two thousand and five hundred clinically significant *P. aeruginosa* isolates will be selected from hospital collections. Only centres that have storage isolates from at least two years prior the initiation of the study (i.e. 2015-2017) will be eligible to participate in the study.

From each centre, consecutive isolates deemed clinically significant according to local records will be chosen starting from the most recent and working backwards. Isolates from the same

patient will be excluded from the study. Data from Public Health England indicates that two to five cases of *P. aeruginosa* bacteraemia are reported per month from large centres across the country[19]. Taking into consideration that these data only accounts from bacteraemia isolates, it is expected that each participant laboratory will provide a minimum of 50 isolates; no more than 100 isolates will be collected from a single laboratory in order to minimise sample size variability across participant centres.

Up to 30% of the isolates included in the study will have been isolated from cystic fibrosis patients. As not all participant centres will specialized in CF management and treatment, no particular proportion of these isolates is required by the individual centres. It is anticipated that some centres will contribute more CF isolates than others, but no more than 50% CF isolates per centre to allow for non-CF isolates to be included in the study. If this threshold is reached at a centre then any further CF isolates should be excluded during the consecutive search until the percentage of CF isolates falls below 50% of the cumulative total; this should be repeated as required until the maximum number of total isolates is achieved.

- Identification of isolates

Identification to species level of all isolates included in the study will be confirmed at the local laboratories using the available methodology in accordance with the manufacturer recommendations and their standard operating procedures. MALDI-ToF MS is the preferred methodology for species identification at the local laboratories. However, other validated methodologies are allowed for those laboratories in which MALDI-ToF MS is not available. In such cases, isolates will be re identified at the Central Testing Laboratory by MALDI-ToF MS.

3.4 Susceptibility testing

Antimicrobial susceptibility testing will be performed by disc diffusion following EUCAST methodology and standards. [15]

Briefly, a standardized inoculum of 0.5 McFarland is prepared in saline from a freshly grown pure culture of each isolate using the direct colony suspension method. The inoculum is them evenly spread in Mueller–Hinton agar plates ensuring that there are no gaps between streaks and the antibiotic discs are applied to the surface of the inoculated agar plate. This can be done using a disc dispenser. The number of discs on a plate should be limited to avoid overlapping of zones and interference between agents (i.e maximum of six antibiotic discs per plate). Plates are incubated at 35±1°C in air for 16-20 hours.[15]

The zone edge should be read at the point of complete inhibition as judged by the naked eye and results are to be interpreted according to EUCAST criteria. [15,20]

P. aeruginosa ATCC 27853 will be used as quality control strains at every centre to monitor the performance of the test.[15]

Detailed EUCAST disc diffusion method (*Antimicrobial susceptibility testing EUCAST disk diffusion method. Version 6.0. January 2017*) and EUCAST breakpoint tables (*EUCAST breakpoint tables for interpretation of MICs and zone diameters Version 7.1. March 2017*) can be found in section “12- Attachments” of this document.

Antimicrobial Agents for Testing			
Antibiotic	Disc content (µg)	Antibiotic	Disc content (µg)
Ceftolozane/tazobactam	30-10	Aztreonam	30
Piperacillin/tazobactam	30-6	Amikacin	30
Ceftazidime	10	Gentamicin	10
Imipenem	10	Tobramicin	10
Meropenem	10	Ciprofloxacin	5

3.5 Transport and storage of isolates

Collecting laboratories will send the isolates to the Central Testing Laboratory in suitable media (i.e on agar slope), and in compliance with prevailing transport regulations.

On receipt at the Central Testing Laboratory, the isolates will be stored frozen at -80°C and be available for future research.

3.6 Quality assurance

All participant centres must have a UK accredited laboratory where all study analysis will be carried out (re-identification of *P. aeruginosa* isolates and antimicrobial susceptibility testing using the disc diffusion method). Isolates found not to be *P. aeruginosa* will be excluded as per the study criteria.

- Internal quality control

As per EUCAST recommendations, *P. aeruginosa* ATCC 27853 will be used as the quality control strain at every centre to monitor the performance of the test. Either *E. coli* ATCC 35218 or *K. pneumoniae* ATCC 700603 will be used to check the inhibitor component. These control strains will be tested every day that study isolates are analysed.[21] Internal quality control failure will invalidate the results and require repeat analysis.

- External quality control

The susceptibility of at least 10% of the isolates per centre will be re-assessed at the Central Testing Laboratory for reproducibility and external quality control on an isolate number exactly divisible by 10, in line with UKAS (United Kingdom Accreditation Service) guidance. In addition, all isolates with a zone diameter for ceftolozane/tazobactam within ±1mm the breakpoint (i.e.23-25mm), as per each referring centre's susceptibility testing results, will also be included in the external quality control study.

Those isolates with a different inhibition zone (±1mm to allow for operator variability) at the external quality control testing, compared to the results reported by the individual centres, will be tested in triplicate at the Central Testing Laboratory. Results will be reported as observed.

These isolates will be identified to species level by MALDI-ToF and susceptibility tested as described in the "Susceptibility testing" section above. In addition, all isolates that have been identified at the local laboratories using a methodology other than MALDI-ToF MS will be re identified by MALDI-ToF MS at the Central Testing Laboratory.

3.7 Data collection and handling

There will be suitable safeguards to ensure that data is entered into the study records accurately, maintained securely, and disseminated only to authorised recipients. In this regards, data will be collated in an Excel spread sheet with range check. Conditional formatting will be used to account for incorrect data entry.

- All participant laboratories (including the Central Testing Laboratory)

For each isolate, the following information will be recorded in a study sheet (Excel) by the participant laboratories. Study sheets will be provided by the Central Testing Laboratories to all centres.

Background information	
Demographic data	Phenotypic characteristics of isolates
Date of specimen collection Type of specimen (e.g. blood, CSF, BAL, urine, etc) Site of infection Location of patient/ward Cystic Fibrosis (Yes/No)	Mucoid Non-Mucoid

Study results
Per isolate
Isolate ID
Date in which testing was performed
Inoculum size (McFarland turbidity standard)
Incubation time and temperature
Inhibition zone diameter (mm) per isolate/per antibiotic
Inhibition zone diameter (mm) for control strain/ per antibiotic (internal quality control)
Summary
Percentage of susceptible/resistant isolates per antibiotic (overall)
Percentage of susceptible/resistant isolates per antibiotic/per ward
Percentage of susceptible/resistant isolates per antibiotic/per sample source
Percentage of susceptible/resistant isolates per antibiotic in CF and non-CF isolates
Percentage of susceptible/resistant isolates per antibiotic in carbapenem R and carbapenem S isolates

It is intended that each participant laboratory will send the complete and final data and isolates to the Central Testing Laboratory by the 1st of March 2018.

- Central Testing Laboratory

Data from the external quality control assays will be collected by the Central Testing Laboratory only. Susceptibility results from the referring and central laboratories will be compared for the same isolate.

Data from participating laboratory	**Data from Central Testing Laboratory**
Isolate ID	Isolate ID
Date in which testing was performed	Date in which testing was performed
Inoculum size (McFarland turbidity standard)	Inoculum size (McFarland turbidity standard)
Incubation time and temperature	Incubation time and temperature
Inhibition zone diameter (mm) per isolate/per antibiotic	Inhibition zone diameter (mm) per isolate/per antibiotic
Inhibition zone diameter (mm) for control strain/ per antibiotic (internal quality control)	Inhibition zone diameter (mm) for control strain/ per antibiotic (internal quality control)

The Central Testing Laboratory will produce a study summary sheet which will collate data from individual laboratories as well as an overall analysis of all isolates included in the study.

Study results (all participant centres)
Percentage of susceptible/resistant isolates per antibiotic/per centre
Percentage of susceptible/resistant isolates per antibiotic-all centres
Percentage of susceptible/resistant isolates per antibiotic/per ward-all centres
Percentage of susceptible/resistant isolates per antibiotic/per sample source-all centres
Percentage of susceptible/resistant isolates per antibiotic in CF and non-CF isolates-all centres
Percentage of susceptible/resistant isolates per antibiotic in carbapenem R and carbapenem S isolates
Zone distributions (graphical)

3.8 Study Population

Please refer to section "3.3-Isolates" above for detailed description of the isolates.

3.9 Inclusion Criteria

- Clinically significant consecutive *P. aeruginosa* isolates
- Isolates collected during the study period.

3.10 Exclusion Criteria

- Participating centres are asked to provide at least 50 clinical significant *P. aeruginosa.* Laboratories that are unable to meet this criterion will be excluded from the study.
- Laboratories that are unable to perform disc susceptibility testing will be excluded from the study.
- Isolates not identified as *P. aeruginosa.*
- Additional isolates from a patient whose isolate has already been included.

3.11 Stratification

Isolates will be stratified on the basis of their susceptibility profile to carbapenems and whether they are from a patient with CF. These two criteria will be accounted for as part of a sub analysis of the results.

4 Variables and Epidemiological Measurements

4.1 Outcomes

- *To evaluate the in vitro activity of ceftolozane/tazobactam and other commonly used antipseudomonal antibiotics against geographically spread P. aeruginosa isolates in the UK.*

Antimicrobial susceptibility testing will be performed by disc diffusion following EUCAST methodology and standards. The inhibition zone for all antibiotics (see section 3 Methodology for a complete list of antibiotics analysed), expressed in mm, will be measured and captured in the study sheet at each centre.

Results will be presented as percentage of susceptible/resistant isolates per centre and overall, and distribution of inhibition zone per antibiotic per centre and overall.

No statistical analysis will be required.

- *To provide centres with the opportunity to generate local susceptibility data that will guide appropriate antimicrobial treatment for P. aeruginosa.*

Each participant centre will perform susceptibility testing of their own *P. aeruginosa* isolates. Following susceptibility testing, results will be analysed and presented as percentage of susceptible/resistant isolates and distribution of inhibition zone per antibiotic. These data will be used to further understanding of appropriate antimicrobial options for the treatment of *P. aeruginosa* infections.

- *To assess the inter-laboratory reproducibility of results using the EUCAST disc susceptibility methodology.*

A selection of isolates per centre will be re analysed at the Central Testing Laboratory for external quality control purposes. Those isolates with a different inhibition zone at the external quality control testing, compared to the results reported by the individual centres, will be tested in triplicate at the Central Testing Laboratory. Results will be reported as observed.

4.2 Covariates

The section does not apply as this is an *in vitro* study.

5 Study Procedures

5.1 General Informed Consent

The section does not apply as this is an *in vitro* study.

6 Safety Reporting and Related Procedures

Adverse Event Reporting

ADVERSE EVENT REPORTING

This is an *in vitro* study using bacterial isolates based on secondary use of bacterial isolates collected for other purposes. No administration of any therapeutic or prophylactic agent is required in this protocol. No reporting of individual adverse events to regulatory agencies is planned for this *in vitro* study because there is no access to individual patient/subject records.

Any relevant safety information will be summarized in the appropriate Periodic Safety Update Report (PSUR)/Periodic Benefit Risk Evaluation Report (PBRER) and/or Development Safety Update Reports (DSUR) if required. Pre-specified health outcomes of interest, including any that qualify as adverse events, will be summarized as part of any interim analysis (including safety analysis, if required) and in the final study report, which will be provided to regulatory agencies by the sponsor as required.

If an investigator elects to spontaneously report any suspected adverse reactions, they should be reported via fax to Merck AER Mailbox FAX #215-661-6229 (US), or toll-free fax 1-800-547-5552 (ex-US and US availability), in English using an AE form (**attached**) for reporting to worldwide regulatory agencies as appropriate.

7 Statistical Analysis Plan

7.1 Statistical Methods

In this descriptive *in vitro* analysis, data will be collated in an Excel spread sheet with range check for each antibiotic inhibition zone. Conditional formatting will be used to account for incorrect data entry.

7.1.1 Primary Objective(s):

- To evaluate the *in vitro* activity of ceftolozane/tazobactam and other commonly used antipseudomonal antibiotics against geographically spread *P. aeruginosa* isolates in the UK.

The *in vitro* activity of ceftolozane/tazobactam and other antibiotics in the study will be expressed as inhibition zone diameter (mm) and summarised as a table for all isolates and by centre. Distribution of inhibition zone diameters will be represented in a graph.

Percentage of susceptible and resistant isolates per antibiotic will be calculated considering EUCAST break points. Subanalysis will include susceptible and resistant isolates per antibiotic and ward, sample source, CF/ non-CF and carbapenem R/S.

7.1.2 Secondary Objective(s):

- To provide centres with the opportunity to generate local susceptibility data that will guide appropriate antimicrobial treatment for *P. aeruginosa.*

Each participant centre will collate and analyse susceptibility data for their own isolates for ceftolozane/tazobactam and other antibiotics. Susceptibility results will be expressed as inhibition zone diameter (mm) and summarised as a table for all isolates. Distribution of inhibition zone diameters will be represented in a graph.

Percentage of susceptible and resistant isolates per antibiotic will be calculated considering EUCAST break points. Subanalysis will include susceptible and resistant isolates per antibiotic and ward, sample source, CF/ non-CF and carbapenem R/S.

- To ensure disc susceptibility testing is performed both accurately and consistently across centres and to assess the inter-laboratory reproducibility of results using the EUCAST disc susceptibility methodology.

Data from the external quality control assays will be collected by the Central Testing Laboratory. Susceptibility results from the referring and central laboratories will be summarised in a table and compared for the same isolate.

7.2 Bias

- Selection bias

Different criteria for storing *P.aeruginosa* isolates may have been applied at each participant centre.

7.2.1 Methods to Minimize Bias

Consecutive isolates from individual centres will reduce the likelihood of centres picking specific patient or sample types. Sub-group analysis will also help analyse the characteristics of specific isolates based on antibiogram and sample type.

7.2.3 Limitations

The study is a mostly retrospective analysis of unselected stored bacterial isolates from multiple laboratories with heterogeneous patient populations and differing criteria for storing isolates. It is therefore inherently susceptible to selection bias as above. However, it will provide a real-world analysis of clinically significant isolates and allows for sub-group analysis.

Inter-laboratory variability in susceptibility testing performance is well established when performing phenotypic analysis of bacterial isolates; results for cystic fibrosis isolates are especially prone to this issue. However, the external quality control will assess any significant variation.

7.3 Sample Size and Power Calculations

The number of participant centres has been decided in line with other well-stablished national susceptibility programmes and centres have been selected to provide a good geographical spread throughout the United Kingdom. The majority of the centres will be tertiary or teaching hospitals, centres with large ITUs and CF units where pseudomonal infections are more likely to occur.

Based on surveillance data from the UK, it is anticipated that each participant laboratory will be able to provide at least 50 *P. aeruginosa* isolates. A cap of 100 isolates has been decided in order to minimise sample size variability across participant centres.

See section *3.2 Centres* and *3.3 Isolates* for further details

8 Administrative and Regulatory Details

8.1 Confidentiality

8.1.1 Confidentiality of Data

By signing this protocol, the investigator affirms to the Sponsor that information furnished to the investigator by the Sponsor will be maintained in confidence, and such information will be divulged to the Institutional Review Board, Ethics Review Committee or similar or expert committee; affiliated institution and employees, only under an appropriate understanding of confidentiality with such board or committee, affiliated institution and employees. Data generated by this study will be considered confidential by the investigator, except to the extent that it is included in a publication as provided in the Publications section of this protocol.

8.1.2 Confidentiality of Subject Records

Only bacterial isolates will be included and analysed in the study. No human tissue will be collected, stored or analysed and no patient data will be recorded.

By signing this protocol, the investigator agrees that the Sponsor (or Sponsor representative), Institutional Review Board/Independent Ethics Committee (IRB/IEC), or Regulatory Agency representatives may consult and/or copy study documents in order to verify worksheet/case report form data. By signing the consent form, the subject agrees to this process. If study documents will be photocopied during the process of verifying worksheet/case report form information, the subject will be identified by unique code only; full names/initials will be masked prior to transmission to the Sponsor.

By signing this protocol, the investigator agrees to treat all subject data used and disclosed in connection with this study in accordance with all applicable privacy laws, rules and regulations.

8.1.3 Confidentiality of Investigator Information

By signing this protocol, the investigator recognizes that certain personal identifying information with respect to the investigator, and all subinvestigators and study site personnel, may be used and disclosed for study management purposes, as part of a regulatory submissions, and as required by law. This information may include:

- name, address, telephone number and e-mail address;
- hospital or clinic address and telephone number;
- curriculum vitae or other summary of qualifications and credentials; and
- other professional documentation.

Consistent with the purposes described above, this information may be transmitted to the Sponsor, and subsidiaries, affiliates and agents of the Sponsor, in your country and other

countries, including countries that do not have laws protecting such information. Additionally, the investigator's name and business contact information may be included when reporting certain serious adverse events to regulatory agencies or to other investigators. By signing this protocol, the investigator expressly consents to these uses and disclosures.

If this is a multicenter study, in order to facilitate contact between investigators, the Sponsor may share an investigator's name and contact information with other participating investigators upon request.

8.2 Compliance with Financial Disclosure Requirements

Financial Disclosure requirements are outlined in the US Food and Drug Administration Regulations, Financial Disclosure by Clinical Investigators (21 CFR Part 54). It is the Sponsor's responsibility to determine, based on these regulations, whether a request for Financial Disclosure information is required. It is the investigator's/subinvestigator's responsibility to comply with any such request.

The investigator/subinvestigator(s) agree, if requested by the Sponsor in accordance with 21 CFR Part 54, to provide his/her financial interests in and/or arrangements with the Sponsor to allow for the submission of complete and accurate certification and disclosure statements. The investigator/subinvestigator(s) further agree to provide this information on a Certification/Disclosure Form, commonly known as a financial disclosure form, provided by the Sponsor or through a secure password-protected electronic portal provided by the Sponsor. The investigator/subinvestigator(s) also consent to the transmission of this information to the Sponsor in the United States for these purposes. This may involve the transmission of information to countries that do not have laws protecting personal data.

8.3 Compliance with Law, Audit and Debarment

By signing this protocol, the investigator agrees to conduct the study in an efficient and diligent manner and in conformance with this protocol; generally accepted standards of Good Pharmacoepidemiology Practice and all applicable federal, state and local laws, rules and regulations relating to the conduct of the study.

The investigator also agrees to allow monitoring, audits, Institutional Review Board/Independent Ethics Committee review and regulatory agency inspection of study-related documents and procedures and provide for direct access to all study-related source data and documents.

The investigator agrees not to seek reimbursement from subjects, their insurance providers or from government programs for procedures included as part of the study reimbursed to the investigator by the Sponsor.

The Investigator shall prepare and maintain complete and accurate study documentation in compliance with Good Pharmacoepidemiology Practice, standards and applicable federal, state and local laws, rules and regulations; and, for each subject participating in the study, provide all data, and, upon completion or termination of the study, submit any other reports to the Sponsor as required by this protocol or as otherwise required pursuant to any agreement with the Sponsor.

Study documentation will be promptly and fully disclosed to the Sponsor by the investigator upon request and also shall be made available at the investigator's site upon request for inspection, copying, review and audit at reasonable times by representatives of the Sponsor or any regulatory agencies. The investigator agrees to promptly take any reasonable steps that are requested by the Sponsor as a result of an audit to cure deficiencies in the study documentation and worksheets/case report forms.

The investigator must maintain copies of all documentation and records relating to the conduct of the study in accordance with their institution's records retention schedule which is compliant with all applicable regional and national laws and regulatory requirements. If an institution does not have a records retention schedule to manage its records long-term, the investigator must maintain all documentation and records relating to the conduct of the study for 5 years after final report or first publication of study results, whichever comes later, per GPP guidelines. This documentation includes, but is not limited to, the protocol, worksheets/case report forms, advertising for subject participation, adverse event reports, subject source data, correspondence with regulatory authorities and IRBs/ERCs, consent forms, investigator's curricula vitae, monitor visit logs, laboratory reference ranges, laboratory certification or quality control procedures and laboratory director curriculum vitae. All study documents shall be made available if required by relevant regulatory authorities. The investigator must consult with the Sponsor prior to discarding study and/or subject files.

The investigator will promptly inform the Sponsor of any regulatory agency inspection conducted for this study.

Persons debarred from conducting or working on studies by any court or regulatory agency will not be allowed to conduct or work on this Sponsor's studies. The investigator will immediately disclose in writing to the Sponsor if any person who is involved in conducting the study is debarred or if any proceeding for debarment is pending or, to the best of the investigator's knowledge, threatened.

In the event the Sponsor prematurely terminates a particular study site, the Sponsor will promptly notify that site's IRB/IEC.

According to European legislation, a Sponsor must designate an overall coordinating investigator for a multi-center study (including multinational). When more than one study site is open in an EU country, Merck, as the Sponsor, will designate, per country, a national principal coordinator (Protocol CI), responsible for coordinating the work of the principal investigators at the different sites in that Member State, according to national regulations. For a single-center study, the Protocol CI is the principal investigator. In addition, the Sponsor must designate a principal or coordinating investigator to review the study report that summarizes the study results and confirm that, to the best of his/her knowledge, the report accurately describes the conduct and results of the study in the study's final report. The Sponsor may consider one or more factors in the selection of the individual to serve as the Protocol CI and or CSR CI (e.g., availability of the CI during the anticipated review process, thorough understanding of study methods, appropriate enrollment of subject cohort, timely achievement of study milestones). The Protocol CI must be a participating study investigator.

8.4 Quality Management System

By signing this protocol, the Sponsor agrees to be responsible for implementing and maintaining a quality management system with written development procedures and functional area standard operating procedures (SOPs) to ensure that studies are conducted and data are generated, documented, and reported in compliance with the protocol, accepted standards of Good Pharmacoepidemiology Practice, and all applicable federal, state, and local laws, rules and regulations relating to the conduct of the study.

8.5 Data Management

The investigator or qualified designee is responsible for recording and verifying the accuracy of subject data. By signing this protocol, the investigator acknowledges that his/her electronic

signature is the legally binding equivalent of a written signature. By entering his/her electronic signature, the investigator confirms that all recorded data have been verified as accurate.

For an outsourced study the institutional policies of the vendor should be followed for development of data management plans. However, the vendor should ensure compliance with Good Pharmacoepidemiology Practice, and all applicable federal, state, and local laws, rules and regulations relating to the conduct of the study.

9 Publications

It is expected that the initial study results will be presented at the Federation of Infection Societies Annual Conference 2018. Abstract should be prepared for submission in September 2018.

10 References

1. Vincent, J., Marshall, J., Anzueto, A., Martin, C. D. & Gomersall, C. International study of the prevalence and outcomes of infection in Intensive Care Units. *JAMA* **302,** 2323–2329 (2009).
2. Lopez-Casusape, C., Rojo-Molinero, E., Macia, M. & Oliver, A. The problems of antibiotic resistance in cystic fibrosis and solutions. *Expert Rev Respir Med* 73–88 (2015).
3. Zamorano, Carlos, J., Pérez, L., L., J., Ge, Y. & Oliver, A. Activity of a new antipseudomonal cephalosporin, CXA-101 (FR264205), against carbapenem-resistant and multidrug-resistant Pseudomonas aeruginosa clinical strains. *Antimicrob. Agents Chemother.* **54,** 846–851 (2010).
4. Cystic Fibrosis Trust. UK Cystic Fibrosis Registry Annual Data Report 2016. 60 (2016).
5. Mesaros, N. *et al.* Pseudomonas aeruginosa: Resistance and therapeutic options at the turn of the new millennium. *Clin. Microbiol. Infect.* **13,** 560–578 (2007).
6. Livermore, D. M. *et al.* Activity of ceftolozane/tazobactam against surveillance and 'problem' Enterobacteriaceae, Pseudomonas aeruginosa and non-fermenters from the British Isles. *J. Antimicrob. Chemother.* 2278–2289 (2017). doi:10.1093/jac/dkx136
7. Nathwani, D., Raman, G., Sulham, K., Gavaghan, M. & Menon, V. Clinical and economic consequences of hospital-acquired resistant and multidrug-resistant Pseudomonas aeruginosa infections: a systematic review and meta-analysis. *Antimicrob. Resist. Infect. Control* **3,** 32 (2014).
8. Zhanel, G. G. *et al.* Ceftolozane/tazobactam: A novel cephalosporin/beta-lactamase inhibitor combination with activity against multidrug-resistant gram-negative bacilli. *Drugs* **74,** 31–51 (2014).
9. Livermore, D. M., Mushtaq, S., Ge, Y. & Warner, M. Activity of cephalosporin CXA-101 (FR264205) against Pseudomonas aeruginosa and Burkholderia cepacia group strains and isolates. *Int. J. Antimicrob. Agents* **34,** 402–406 (2009).
10. Farrell, D. ., Sader, H. S., Mendes, R. E. & Jones, R. N. Ceftolozane / Tazobactam Activity against Intra-Abdominal Pathogens Collected from 41 Medical Centres in Europe , Turkey and Israel (2014). *ECCMID 2016* (2016).
11. Farrell, D. ., Sader, H. S., Mendes, R. E. & Jones, R. N. Antimicrobial Activity of

Ceftolozane / Tazobactam from the PACTS Programme Tested Against Pseudomonas aeruginosa Isolated from Patients Hospitalised in Intensive Care Units in Europe , Turkey and Israel (2013-2014). *ECCMID 2016* (2016).

12. Shortridge, D., Castanheira, M., Duncan, L. & Sader, H. S. Activity of Ceftolozane / Tazobactam and Comparators Tested Against Carbapenem-Nonsusceptible Pseudomonas aeruginosa Isolates. *ID week p1823* 2016 (2016).

13. BSAC. BSAC Resistance Surveillance Project. Available at: http://www.bsacsurv.org/.

14. Jorgensen, J. H. & Ferraro, M. J. Antimicrobial Susceptibility Testing : A Review of General Principles and Contemporary Practices. *Clin. Infect. Dis.* **49,** 1749–1755 (2009).

15. EUCAST. Antimicrobial susceptibility testing EUCAST disk diffusion method - Version 6.0. **0,** 1–22 (2017).

16. Hirsch, E. B. & Tam, V. H. Impact of multidrug-resistant Pseudomonas aeruginosa infection on patient outcomes. *Expert Rev. Pharmacoecon. Outcomes Res.* **10,** 441–51 (2010).

17. Aloush, V., Navon-venezia, S., Seigman-igra, Y., Cabili, S. & Carmeli, Y. Multidrug-Resistant Pseudomonas aeruginosa : Risk Factors and Clinical Impact. *Antimicrob. Agents Chemother.* **50,** 43–48 (2006).

18. EUCAST. *The European Committee on Antimicrobial Susceptibility Testing. Addendum to the EUCAST breakpoint table v. 7.1, June 2017*.

19. Public Health England. Monthly counts of Pseudomonas aeruginosa bacteraemia split by location of onset, by NHS acute trust. (2017). Available at: https://www.gov.uk/government/statistics/p-aeruginosa-bacteraemia-monthly-data-split-by-location-of-onset-by-nhs-trust.

20. SusceptibilityTesting, E. C. on A. European Committee on Antimicrobial Susceptibility Testing Breakpoint tables for interpretation of MICs and zone diameters European Committee on Antimicrobial Susceptibility Testing Breakpoint tables for interpretation of MICs and zone diameters. *http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_5.0_Breakpoint_Table_01.pdf* 0–77 (2017).

21. EUCAST. The European Committee on Antimicrobial Susceptibility Testing. Routine and extended internal quality control for MIC determination and disk diffusion as recommended by EUCAST. Version 7.0, 2017. http://www.eucast.org. **7,** 0–14 (2017).

11 Attachments

- Antimicrobial susceptibility testing EUCAST disk diffusion method - Version 6.0. **0,** 1–22 (2017).
- Addendum to the EUCAST breakpoint table v. 7.1, The European Committee on Antimicrobial Susceptibility Testing. June 2017.
- European Committee on Antimicrobial Susceptibility Testing Breakpoint tables for interpretation of MICs and zone diameters 2017

12 SIGNATURES

Sponsor's Representative

TYPED NAME	SIGNATURE	DATE
_____	_____	_____

Investigator

I agree to conduct this study in accordance with the design outlined in this protocol and to abide by all provisions of this protocol (including other manuals and documents referenced from this protocol); deviations from the protocol are acceptable only with a mutually agreed upon protocol amendment. I agree to conduct the study in accordance with generally accepted standards of Good Pharmacoepidemiology Practice. I also agree to report all information or data in accordance with the protocol and, in particular, I agree to report any serious adverse experiences as defined in Section 6 – Safety Reporting and Related Procedures. I understand that information that identifies me will be used and disclosed as described in the protocol, and that such information may be transferred to countries that do not have laws protecting such information. Since the information in this protocol is confidential, I understand that its disclosure to any third parties, other than those involved in approval, supervision, or conduct of the study is prohibited. I will ensure that the necessary precautions are taken to protect such information from loss, inadvertent disclosure, or access by third parties.

TYPED NAME	SIGNATURE	DATE
_____	_____	_____

EUCAST EUROPEAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING

European Society of Clinical Microbiology and Infectious Diseases

Antimicrobial susceptibility testing

EUCAST disk diffusion method

Version 6.0

January 2017

Contents

Changes from previous version (v. 5.0)

Section	Change
2.2	Information added on Petri dish dimensions.
2.3	Information added on drying of plates.
2.4	Recommendation changed for storage of in-house prepared plates.
2.7	Clarification on drying of agar plates.
Table 1	*Aerococcus sanguinicola* and *urinae* and *Kingella kingae* added.
3.2, 3.3	Information added on inoculum preparation.
3.3.2	Clarification on the use of McFarland 0.5 standards.
3.4	Summary of 15-15-15 minute rule added (see footnote 1).
Table 2	Information on commercial McFarland standards removed.
4.1	Information added on allowing the plates to reach room temperature before inoculation.
4.3, 4.3.1, 4.3.2	Information added on inoculation of agar plates for Gram-negative and Gram-positive bacteria.
4.4	Information added on inoculation of more than one agar plate.
4.5, 4.5.1	Information added on inoculation techniques.
4.6	Summary of 15-15-15 minute rule added (see footnote 1).
5.3	Time limit on application of disks added. Summary of 15-15-15 minute rule added (see footnote 1).
5.5.2, 5.5.3, 5.5.5	Information added on handling and storage of antimicrobial disks.
6.1	Information added on inverting plates. Summary of 15-15-15 minute rule added (see footnote 1).
6.2	Information added on stacking plates in incubators.
6.3.1	Clarification on incubation time.
Table 3	Time interval for prolonged incubation of *Corynebacterium* spp. corrected.
Table 3	*Aerococcus sanguinicola* and *urinae* and *Kingella kingae* added.
8.5, 8.5.1	Clarification on reading of zones and the use of automated zone readers.
8.8	Information on EUCAST Reading Guide added.
8.9.1	Clarification on reading of zones in case of double zones or distinct colonies within zones.
8.9.2	Clarification on reading of trimethoprim-sulfamethoxazole zones for *Stenotrophomonas maltophilia.*
8.9.3	Reading instructions for Enterobacteriaceae with ampicillin-sulbactam and amoxicillin-clavulanic acid added.
8.9.6	Reading instruction for benzylpenicillin updated.

Section	Change
8.9.10	Clarification on how to differentiate between haemolysis and growth when reading inhibition zones.
8.9.11	Instructions for reading of fosfomycin zones for *Escherichia coli* added.
9.1.1	Information added on control of the inhibitor component of β-lactam-β-lactamase inhibitor combination disks.
9.3	Information added on subculturing of control strains.
9.4.1	Information added on how to use EUCAST QC targets and ranges.
9.5	Recommendation changed for quality control frequency.
Table 4	Information added on characteristics for *Escherichia coli* ATCC 35218.
Table 4	*Klebsiella pneumoniae* ATCC 700603 added.
Table 4	*Haemophilus influenzae* NCTC 8468 removed.
Table 5	Characteristics for *Haemophilus influenzae* ATCC 49766 reworded.

Abbreviations and terminology

ATCC	American Type Culture Collection http://www.atcc.org
CCUG	Culture Collection University of Göteborg http://www.ccug.se
CECT	Colección Española de Cultivos Tipo. http://www.cect.org
CIP	Collection de Institut Pasteur http://www.cabri.org/CABRI/srs-doc/cip_bact.info.html
DSM	Bacterial cultures from Deutsche Stammsammlung für Mikroorganismen und Zellkulturen (DSMZ) have DSM numbers https://www.dsmz.de/
ESBL	Extended Spectrum β-Lactamase
EUCAST	European Committee on Antimicrobial Susceptibility Testing http://www.eucast.org
MH	Mueller-Hinton agar
MH-F	Mueller-Hinton agar Fastidious organisms (MH supplemented with 5% defibrinated horse blood and 20 mg/L β-NAD)
MRSA	Methicillin Resistant *Staphylococcus aureus* (with *mecA* or *mecC* gene)
NCTC	National Collection of Type Cultures http://www.hpacultures.org.uk
ß-NAD	ß-Nicotinamide Adenine Dinucleotide
Saline	A 0.85% solution of NaCl in water (8.5 g/L)

1 Introduction

Disk diffusion is one of the oldest approaches to antimicrobial susceptibility testing and remains one of the most widely used antimicrobial susceptibility testing methods in routine clinical laboratories. It is suitable for testing the majority of bacterial pathogens, including the more common fastidious bacteria, is versatile in the range of antimicrobial agents that can be tested and requires no special equipment.

In common with several other disk diffusion techniques, the EUCAST method is a standardised method based on the principles defined in the report of the International Collaborative Study of Antimicrobial Susceptibility Testing, 1972, and the experience of expert groups worldwide.

The zone diameter breakpoints in the EUCAST method are calibrated to the harmonised European MIC breakpoints that are published by EUCAST and are freely available from the EUCAST website (http://www.eucast.org).

As with all standardised methods, the described technique must be followed without modification in order to produce reliable results.

2 Preparation and storage of media

2.1 Prepare Mueller-Hinton (MH) agar according to the manufacturer's instructions, with supplementation for fastidious organisms as indicated in **Table 1**. Preparation and addition of supplements are described in detail at http://www.eucast.org.

2.2 The medium should have a level depth of 4 ± 0.5 mm (approximately 25 mL in a 90 mm circular plate, 31 mL in a 100 mm circular plate, 71 mL in a 150 mm circular plate, 40 mL in a 100 mm square plate). Ascertain that a correct volume, based on the true dimensions of the Petri dish in use, is calculated. Plate dimensions may differ between manufacturers.

2.3 The surface of the agar should be dry before use. No drops of water should be visible on the surface of the agar or inside the lid. If necessary, dry plates either at 20-25°C overnight, or at 35°C, with the lid removed, for 15 min. Do not over-dry plates.

2.4 Store plates prepared in-house at 4-8°C.

2.5 For plates prepared in-house, plate drying, storage conditions and shelf life should be determined as part of the laboratory quality assurance programme.

2.6 Commercially prepared plates should be stored as recommended by the manufacturer and used within the labelled expiry date.

2.7 For agar plates (commercially or in-house prepared) stored in plastic bags or sealed containers, it may be necessary to dry the plates prior to use (see section 2.3). This is to avoid excess moisture, which may result in problems with fuzzy zone edges and/or haze within zones.

Table 1	**Media for antimicrobial susceptibility testing**
Organism	**Medium**
Enterobacteriaceae	MH agar
Pseudomonas spp.	MH agar
Stenotrophomonas maltophilia	MH agar
Acinetobacter spp.	MH agar
Staphylococcus spp.	MH agar
Enterococcus spp.	MH agar
Streptococcus groups A, B, C and G	MH-F agar[1]
Streptococcus pneumoniae	MH-F agar[1]
Viridans group streptococci	MH-F agar[1]
Haemophilus influenzae	MH-F agar[1]
Moraxella catarrhalis	MH-F agar[1]
Listeria monocytogenes	MH-F agar[1]
Pasteurella multocida	MH-F agar[1]
Campylobacter jejuni and *coli*	MH-F agar[1] (see Appendix A)
Corynebacterium spp.	MH-F agar[1]
Aerococcus sanguinicola and *urinae*	MH-F agar[1]
Kingella kingae	MH-F agar[1]
Other fastidious organisms	Pending

[1] MH + 5% mechanically defibrinated horse blood + 20 mg/L β-NAD

3 Preparation of inoculum

3.1 Use the direct colony suspension method to make a suspension of the organism in saline to the density of a McFarland 0.5 turbidity standard (**Table 2**), approximately corresponding to 1-2 x 10^8 CFU/mL for *Escherichia coli*.

The direct colony suspension method is appropriate for all organisms, including fastidious organisms in **Table 1**.

3.2 Use a sterile loop or a cotton swab to pick colonies from an overnight culture on non-selective media. Use several morphologically similar colonies (when possible) to avoid selecting an atypical variant. Suspend the colonies in saline and mix to an even turbidity.

3.3 Adjust the density of the organism suspension to McFarland 0.5 by adding saline or more bacteria. A denser inoculum will result in reduced zones of inhibition and a decreased inoculum will have the opposite effect.

3.3.1 It is recommended that a photometric device is used to adjust the density of the suspension. The photometric device must be calibrated against a McFarland 0.5 standard according to the manufacturer's instruction.

3.3.2 Alternatively, the density of the suspension can be compared visually to a McFarland 0.5 turbidity standard. To aid comparison, compare the test and standard against a white background with black lines.

3.3.3 *Streptococcus pneumoniae* is, preferably, suspended from a blood agar plate to the density of a McFarland 0.5 standard. When *Streptococcus pneumoniae* is suspended from a chocolate agar plate, the inoculum must be equivalent to a McFarland 1.0 standard.

3.4 The suspension should optimally be used within 15 min[1] and always within 60 min of preparation.

[1] Part of the 15-15-15 minute rule: use the inoculum suspension within 15 minutes of preparation, apply disks within 15 minutes of inoculation and incubate plates within 15 minutes of disk application.

Table 2	Preparation of McFarland 0.5 turbidity standard
1	Add 0.5 mL of 0.048 mol/L $BaCl_2$ (1.175% w/v $BaCl_2 \cdot 2H_20$) to 99.5 mL of 0.18 mol/L (0.36 N) H_2S0_4 (1% v/v) and mix thoroughly.
2	Check the density of the suspension in a spectrophotometer with a 1 cm light path and matched cuvettes. The absorbance at 625 nm should be in the range 0.08 to 0.13.
3	Distribute the suspension into tubes of the same size as those used for bacterial inoculum suspensions. Seal the tubes.
4	Store sealed standards in the dark at room temperature.
5	Mix the standard thoroughly on a vortex mixer immediately before use.
6	Renew standards or check their absorbance after storage for 6 months.

4	Inoculation of agar plates
4.1	Make sure that agar plates are at room temperature prior to inoculation.
4.2	Optimally, use the adjusted inoculum suspension within 15 min[1] of preparation. The suspension must always be used within 60 min of preparation.
4.3	Dip a sterile cotton swab into the suspension.
4.3.1	To avoid over-inoculation of Gram-negative bacteria, remove excess fluid by pressing and turning the swab against the inside of the tube.
4.3.2	For Gram-positive bacteria, do not press or turn the swab against the inside of the tube.
4.4	When inoculating several agar plates with the same inoculum suspension, repeat the procedure in section 4.3 for each agar plate.
4.5	Plates can be inoculated either by swabbing in three directions or by using an automatic plate rotator. Spread the inoculum evenly over the entire agar surface ensuring that there are no gaps between streaks.
4.5.1	For Gram-positive bacteria, take particular care to ensure that there are no gaps between streaks.
4.6	Apply disks within 15 min[1] of inoculation. If inoculated plates are left at room temperature for prolonged periods of time before the disks are applied, the organism may begin to grow, resulting in erroneous reduction in sizes of inhibition zone diameters.

[1] Part of the 15-15-15 minute rule: use the inoculum suspension within 15 minutes of preparation, apply disks within 15 minutes of inoculation and incubate plates within 15 minutes of disk application.

5 Application of antimicrobial disks

5.1 The required disk contents are listed in the Breakpoint and Quality Control Tables at http://www.eucast.org.

5.2 Allow disks to reach room temperature before opening cartridges or containers used for disk storage. This is to prevent condensation, leading to rapid deterioration of some agents.

5.3 Apply disks firmly to the surface of the inoculated agar plate within 15 minutes of inoculation[1]. Disks must be in close and even contact with the agar surface and must not be moved once they have been applied as the initial diffusion of antimicrobial agents from disks is very rapid.

5.4 The number of disks on a plate should be limited to avoid overlapping of zones and interference between agents. It is important that zone diameters can be reliably measured. The maximum number of disks depends on the organism and the selection of disks. Normally 6 and 12 disks are the maximum possible number on a 90 and 150 mm circular plate, respectively.

5.4.1 To be able to detect inducible clindamycin resistance in staphylococci and streptococci, the erythromycin and clindamycin disks must be placed at a distance of 12-20 mm from edge to edge for staphylococci and 12-16 mm from edge to edge for streptococci.

5.5 Loss of potency of antimicrobial agents in disks results in reduced inhibition zone diameters and is a common source of error. The following are essential:

5.5.1 Store disks, including those in dispensers, in sealed containers with a desiccant and protected from light (some agents, including metronidazole, chloramphenicol and the fluoroquinolones, are inactivated by prolonged exposure to light).

5.5.2 Store disk stocks according the manufacturers' instructions. Some agents are more labile than others (*e.g.* amoxicillin-clavulanic acid, cefaclor and carbapenems) and specific recommendations may be available from the manufacturers.

5.5.3 Store working supplies of disks according to the manufacturers' instructions. Once disk containers have been opened, disks should be used within the time limit specified by the manufacturer.

5.5.4 Discard disks on the manufacturer's expiry date shown on the container.

5.5.5 Perform frequent quality control (see Section 9) of working supplies to control that the antimicrobial disks have not lost potency during storage.

[1] Part of the 15-15-15 minute rule: use the inoculum suspension within 15 minutes of preparation, apply disks within 15 minutes of inoculation and incubate plates within 15 minutes of disk application.

6 Incubation of plates

6.1 Invert agar plates and make sure disks do not fall off the agar surface. Incubate plates within 15 min[1] of disk application. If the plates are left at room temperature after disks have been applied, pre-diffusion may result in erroneously large zones of inhibition.

6.2 Stacking plates in the incubator may affect results due to uneven heating. The efficiency of incubators varies and therefore the control of incubation, including appropriate numbers of plates in any one stack, should be determined as part of the laboratory's quality assurance programme. For most incubators, a maximum of five plates per stack is appropriate.

6.3 Incubate plates in the conditions shown in **Table 3**.

6.3.1 Incubation beyond the recommended time limits should not be performed as this may result in growth within inhibition zones and reporting isolates as false resistant.

6.3.2 With glycopeptide susceptibility tests on *Enterococcus* spp. resistant colonies may not be visible until plates have been incubated for 24 h. However, plates may be examined after 16-20 h and any resistance reported, but plates of isolates appearing susceptible must be re-incubated and reread at 24 h.

[1] Part of the 15-15-15 minute rule: use the inoculum suspension within 15 minutes of preparation, apply disks within 15 minutes of inoculation and incubate plates within 15 minutes of disk application.

Table 3	Incubation conditions for antimicrobial susceptibility test plates
Organism	**Incubation conditions**
Enterobacteriaceae	35±1°C in air for 16-20 h
Pseudomonas spp.	35±1°C in air for 16-20 h
Stenotrophomonas maltophilia	35±1°C in air for 16-20 h
Acinetobacter spp.	35±1°C in air for 16-20 h
Staphylococcus spp.	35±1°C in air for 16-20 h
Enterococcus spp.	35±1°C in air for 16-20 h (24 h for glycopeptides)
Streptococcus groups A, B, C and G	35±1°C in 4-6% CO_2 in air for 16-20 h
Streptococcus pneumoniae	35±1°C in 4-6% CO_2 in air for 16-20 h
Viridans group streptococci	35±1°C in 4-6% CO_2 in air for 16-20 h
Haemophilus influenzae	35±1°C in 4-6% CO_2 in air for 16-20 h
Moraxella catarrhalis	35±1°C in 4-6% CO_2 in air for 16-20 h
Listeria monocytogenes	35±1°C in 4-6% CO_2 in air for 16-20 h
Pasteurella multocida	35±1°C in 4-6% CO_2 in air for 16-20 h
Campylobacter jejuni and *coli*	See **Appendix A**
Corynebacterium spp.	35±1°C in 4-6% CO_2 in air for 16-20 h. Isolates with insufficient growth after 16-20 h are re-incubated immediately and inhibition zones read after a total of 40-44 h incubation.
Aerococcus sanguinicola and *urinae*	35±1°C in 4-6% CO_2 in air for 16-20 h. Isolates with insufficient growth after 16-20 h are re-incubated immediately and inhibition zones read after a total of 40-44 h incubation.
Kingella kingae	35±1°C in 4-6% CO_2 in air for 16-20 h. Isolates with insufficient growth after 16-20 h are re-incubated immediately and inhibition zones read after a total of 40-44 h incubation.
Other fastidious organisms	Pending

7 Examination of plates after incubation

7.1 A correct inoculum and satisfactorily streaked plates should result in a confluent lawn of growth.

7.1.1 If individual colonies can be seen, the inoculum is too light and the test must be repeated.

7.2 The growth should be evenly distributed over the agar surface to achieve uniformly circular (non-jagged) inhibition zones.

7.3 Check that inhibition zones for quality control strains are within acceptable ranges (http://www.eucast.org).

8 Measurement of zones and interpretation of susceptibility

8.1 For all agents (unless otherwise stated in section 8.9), the zone edge should be read at the point of complete inhibition as judged by the naked eye with the plate held about 30 cm from the eye.

8.2 Read un-supplemented plates from the back with reflected light and the plate held above a dark background.

8.3 Read supplemented plates from the front with the lid removed and with reflected light.

8.4 Do not use transmitted light (plate held up to light) or a magnifying glass, unless otherwise stated (see section 8.9).

8.5 Measure the inhibition zone diameters to the nearest millimetre with a ruler or a calliper.

8.5.1 If an automated zone reader is used, it must be calibrated to manual reading.

8.6 Interpret zone diameters into susceptibility categories according to the current breakpoint tables at http://www.eucast.org.

8.7 If templates are used for interpreting zone diameters, the plate is placed over the template and zones interpreted according to the EUCAST breakpoints marked on the template. Make certain that the breakpoints used are in accordance with the latest version of the EUCAST breakpoint tables. A program for preparation of templates is freely available from http://bsac.org.uk/susceptibility/template-program.

8.8 Several examples of pictures showing reading of inhibition zone diameters are available in the Reading Guide at http://www.eucast.org.This document also includes reading instructions for specific organism-antimicrobial agent combinations.

8.9 Specific reading instructions:

8.9.1 In case of double zones, or distinct colonies within zones, check for purity and repeat the test if necessary. If cultures are pure, colonies within zones should be taken into account when measuring the diameter.

8.9.2 For trimethoprim and trimethoprim-sulfamethoxazole, faint growth up to the disk may appear due to antagonists in the medium. Such growth should be ignored and the zone diameter measured at the more obvious zone edge.

For *Stenotrophomonas maltophilia* with trimethoprim-sulfamethoxazole, an isolate showing any sign of inhibition zone ≥ the susceptible breakpoint should be reported susceptible. Note that there may be substantial growth within zones. Read as no zone only if there is growth up to the disk and no sign of an inhibition zone.

8.9.3 For Enterobacteriaceae with ampicillin, ampicillin-sulbactam and amoxicillin-clavulanic acid, ignore growth that may appear as a thin film producing an inner zone on some batches of Mueller-Hinton agar.

8.9.4	For *Escherichia coli* with mecillinam, ignore isolated colonies within the inhibition zone.
8.9.5	For *Proteus* spp., ignore swarming and read inhibition of growth.
8.9.6	For *Staphylococcus aureus* with benzylpenicillin, examine the zone edge closely with the plate held up to light (transmitted light). Isolates with inhibition zone diameters ≥ the susceptible breakpoint, but with sharp zone edges should be reported resistant.
8.9.7	When using cefoxitin for the detection of methicillin resistance in *Staphylococcus aureus,* measure the obvious zone, and examine zones carefully in good light to detect colonies within the zone of inhibition. These may be either a contaminating species or the expression of heterogeneous methicillin resistance.
8.9.8	Read linezolid susceptibility tests on staphylococci from the back with the plate held up to light (transmitted light).
8.9.9	For enterococci with vancomycin, examine the zone edge closely with the plate held up to light (transmitted light). Fuzzy zone edges and colonies within zone indicate vancomycin resistance and should be investigated further. Isolates must not be reported susceptible before 24 h incubation.
8.9.10	For haemolytic streptococci, read inhibition of growth and not inhibition of haemolysis. β-Haemolysis is usually free from growth, whereas α-haemolysis and growth usually coincide. Tilt the plate back and forth to better differentiate between haemolysis and growth.
8.9.11	For *Escherichia coli* with fosfomycin, ignore isolates colonies within the inhibition zone and read the outer zone edge.

9 Quality control

9.1 Use the quality control (QC) strains specified in **Table 4** to monitor the performance of the test. Principal recommended control strains are typical susceptible strains, but resistant strains can also be used to confirm that the method will detect resistance mediated by known resistance mechanisms (Extended QC, **Table 5**). QC strains may be purchased from culture collections or from commercial sources.

9.1.1 To control the inhibitor component of β-lactam-β-lactamase inhibitor combination disks, specific β-lactamase-producing strains are recommended (**Table 4**). This should be part of the routine QC. The active component is checked with a susceptible QC strain.

9.2 Store control strains under conditions that will maintain viability and organism characteristics. Storage on glass beads at -70°C in glycerol broth (or commercial equivalent) is a convenient method. Non-fastidious organisms can be stored at -20°C. Two vials of each control strain should be stored, one as an in-use supply and the other as an archive for replenishment of the in-use vial when required.

9.3 Each week, subculture a bead from the in-use vial on to appropriate non-selective media and check for purity. From this pure culture, prepare one subculture on each day of the week. For fastidious organisms that will not survive on plates for five to six days, subculture the strain daily for no more than one week.

When subculturing a control strain, use several colonies to avoid selecting a mutant.

9.4 Check that results for control strains are within acceptable ranges in EUCAST QC Tables at http://www.eucast.org.

9.4.1 In EUCAST quality control tables, both ranges and targets are listed. Repeat testing of EUCAST quality control strains should yield zone diameter values randomly distributed within the recommended ranges. If the number of tests is ≥10, the mean zone diameter should be close to the target value (±1 mm from the target value).

9.5 Use the recommended routine quality control strains to monitor test performance.

Control tests should be set up and checked daily, or at least four times per week for antibiotics which are part of routine panels.

Each day that tests are set up, examine the results of the last 20 consecutive tests. Examine results for trends and for zones falling consistently above or below the target. If two or more of 20 tests are out of range investigation is required.

9.6 In addition to routine QC testing, test each new batch of Mueller-Hinton agar to ensure that all zones are within range.

Aminoglycosides may disclose unacceptable variation in divalent cations in the medium, tigecycline may disclose variation in magnesium, trimethoprim-sulfamethoxazole will show up problems with the thymine content, erythromycin can disclose an unacceptable pH.

Table 4:	Quality control organisms for routine testing	
Organism	**Strain**	**Characteristics**
Escherichia coli	ATCC 25922 NCTC 12241 CIP 7624 DSM 1103 CCUG 17620 CECT 434	Susceptible, wild-type
Escherichia coli	ATCC 35218 NCTC 11954 CIP 102181 DSM 5564 CCUG 30600 CECT 943	TEM-1 ß-lactamase, ampicillin resistant (for control of the inhibitor component of β-lactam-β-lactamase inhibitor combination disks)
Klebsiella pneumoniae	ATCC 700603 NCTC 13368 CCUG 45421 CECT 7787	ESBL-producing strain (SHV-18) (for control of the inhibitor component of β-lactam-β-lactamase inhibitor combination disks)
Pseudomonas aeruginosa	ATCC 27853 NCTC 12934 CIP 76110 DSM 1117 CCUG 17619 CECT 108	Susceptible, wild type
Staphylococcus aureus	ATCC 29213 NCTC 12973 CIP 103429 DSM 2569 CCUG 15915 CECT 794	Weak β-lactamase producer
Enterococcus faecalis	ATCC 29212 NCTC 12697 CIP 103214 DSM 2570 CCUG 9997 CECT 795	Susceptible, wild type
Streptococcus pneumoniae	ATCC 49619 NCTC 12977 CIP 104340 DSM 11967 CCUG 33638	Reduced susceptibility to benzylpenicillin
Haemophilus influenzae	ATCC 49766 NCTC 12975 CIP 103570 DSM 11970 CCUG 29539	Susceptible, wild type
Campylobacter jejuni	ATCC 33560 NCTC 11351 CIP 702 DSM 4688, CCUG 11284	Susceptible, wild type For testing conditions, see Appendix A

Table 5:	Additional quality control organisms for detection of specific resistance mechanisms (extended QC)	
Organism	**Strain**	**Characteristics**
Klebsiella pneumoniae	ATCC 700603 NCTC 13368 CCUG 45421 CECT 7787	ESBL-producing strain (SHV-18)
Staphylococcus aureus	NCTC 12493	*mecA* positive, hetero-resistant MRSA
Enterococcus faecalis	ATCC 51299 NCTC 13379 CIP 104676 DSM 12956 CCUG 34289	High-level aminoglycoside resistant (HLAR) and vancomycin resistant (*vanB* positive)
Haemophilus influenzae	ATCC 49247 NCTC 12699 CIP 104604 DSM 9999 CCUG 26214	Reduced susceptibility to β-lactam agents due to PBP mutations (ß-lactamase negative, ampicillin resistant, BLNAR)

Appendix A

Disk diffusion testing of *Campylobacter jejuni* and *coli*

The following methodology (Table A1) must be adhered to when performing disk diffusion testing of *Campylobacter jejuni* and *coli* according to EUCAST.

Table A1	**Disk diffusion methodology for *Campylobacter jejuni* and *coli***
Medium	Mueller-Hinton agar with 5% defibrinated horse blood and 20 mg/L β-NAD (MH-F) In order to reduce swarming, the MH-F plates should be dried prior to inoculation (at 20-25°C overnight, or at 35°C, with the lid removed, for 15 min).
Inoculum	McFarland 0.5
Incubation	Microaerobic environment 41±1°C 24 hours Incubation should result in confluent growth. Some *C. coli* isolates may not have sufficient growth after 24 h incubation. These are re-incubated immediately and inhibition zones read after a total of 40-48 h incubation. An incubation temperature of 41±1°C was chosen to create favourable conditions for growth of *Campylobacter* spp.
Reading	Standard EUCAST reading instructions are used: Read MH-F plates from the front with the lid removed and with reflected light. Zone edges should be read at the point of complete inhibition as judged by the naked eye with the plate held about 30 cm from the eye.
Quality Control	*Campylobacter jejuni* ATCC 33560

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Addendum (June 2017) to the EUCAST breakpoint table v. 7.1

Breakpoints to be included in EUCAST breakpoint tables v 8.0, January 2018

For ceftolozane-tazobactam zone diameter breakpoints for *Pseudomonas aeruginosa* are lacking in the current EUCAST breakpoint table. EUCAST has decided to publish the zone diameter breakpoints now instead of delaying publication until January 2018.

***Pseudomonas aeruginosa* and ceftolozane-tazobactam**	**MIC breakpoint (mg/L)**		**Disk content (µg)**	**Zone diameter breakpoint (mm)**	
	S ≤	**R >**		**S ≥**	**R <**
EUCAST Breakpoint Table v 7.1	4[1]	4[1]		IP	IP
Revised breakpoints 2017	4[1]	4[1]	**30-10**	24	24

1. For susceptibility testing purposes, the concentration of tazobactam is fixed at 4 mg/L.
IP = In Preparation
Changes from EUCAST Breakpoint Tables v 7.1 is highlighted in yellow.

The quality control target and range for *P. aeruginosa* ATCC 27853 and ceftolozane-tazobactam 30-10 µg are available in EUCAST QC Tables v 7.0, 2017 (http://www.eucast.org/ast_of_bacteria/qc_tables/).

EUCAST Steering Committee, June 7, 2017

European Committee on Antimicrobial Susceptibility Testing

Breakpoint tables for interpretation of MICs and zone diameters

Version 7.1, valid from 2017-03-10

This document should be cited as "The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 7.1, 2017. http://www.eucast.org."

European Committee on Antimicrobial Susceptibility Testing

Breakpoint tables for interpretation of MICs and zone diameters

Version 7.1, valid from 2017-03-10

Notes

1. The EUCASTclinical breakpoints tables contain clinical MIC breakpoints (determined or revised during 2002-2016) and their inhibition zone diameter correlates. The EUCAST breakpoint table version 7.0 includes corrected typographical errors, clarifications, breakpoints for new agents and/or organisms, revised MIC breakpoints and revised and new zone diameter breakpoints. Changes are best seen on screen or on a colour printout since cells containing a change are yellow. New or revised comments are underlined. Removed comments are shown in strikethrough font style.

2. PK/PD (Non-species related) breakpoints are listed separately on the last page.

3. Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.

4. Antimicrobial agent names in blue are linked to EUCAST rationale documents. MIC and zone diameter breakpoints in blue are linked to EUCAST MIC and zone diameter distributions, respectively.

5. The document is released as an Excel® file suitable for viewing on screen and as an Acrobat® pdf file suitable for printing. To utilize all functions in the Excel® file, use Microsoft™ original programs only. The Excel® file enables users to alter the list of agents to suit the local range of agents tested. The content of single cells cannot be changed. Hide lines by right-clicking on the line number and choose "hide". Hide columns by right-clicking on the column letter and choose "hide".

6. A zone diameter breakpoint of "S ≥ 50 mm" is an arbitrary "off scale" zone diameter breakpoint corresponding to MIC breakpoint situations where wild type isolates are categorised as intermediate (*i.e.* no fully susceptible isolates exist).

7. In order to simplify the EUCAST tables, the intermediate category is not listed. It is interpreted as values between the S and the R breakpoints. For example, for MIC breakpoints listed as S ≤ 1 mg/L and R > 8 mg/L, the intermediate category is 2-8 (technically >1-8) mg/L, and for zone diameter breakpoints listed as S ≥ 22 mm and R < 18 mm, the intermediate category is 18-21 mm.

8. For *Stenotrophomonas maltophilia* with trimethoprim-sulfamethoxazole, *Staphylococcus aureus* with benzylpenicillin and enterococci with vancomycin, it is crucial to follow specific reading instructions for correct interpretation of the disk diffusion test. For these, pictures with reading examples are included at the end of the corresponding breakpoint table. For general and other specific reading instructions, please refer to the EUCAST Reading Guide.

9. For cefuroxime and fosfomycin there are breakpoints for intravenous and oral administration.

10. By international convention MIC dilution series are based on twofold dilutions up and down from 1 mg/L. At dilutions below 0.25 mg/L, this leads to concentrations with multiple decimal places. To avoid having to use these in tables and documents, EUCAST has decided to use the following format (in bold): 0.125→**0.125**, 0.0625→**0.06**, 0.03125→**0.03**, 0.015625→**0.016**, 0.0078125→**0.008**, 0.00390625→**0.004** and 0.001953125→**0.002** mg/L.

"-" indicates that susceptibility testing is not recommended as the species is a poor target for therapy with the agent. Isolates may be reported as R without prior testing.
"IE" indicates that there is insufficient evidence that the organism or group is a good target for therapy with the agent. An MIC with a comment but without an accompanying S, I or R categorisation may be reported.
NA = Not Applicable
IP = In Preparation

Guidance on reading EUCAST Breakpoint Tables

EUCAST Clinical Breakpoint Tables v. 7.1, valid from 2017-03-10

The intermediate category is not listed but is interpreted as the values between the S and the R breakpoints. If the S and R breakpoints are the same value there is no intermediate category.

Agent A: No intermediate category
Agent B: Intermediate category: 4 mg/L, 23-25 mm
Agent G: Intermediate category: 1-2 mg/L, 24-29 mm

Disk diffusion (EUCAST standardised disk diffusion method)
Medium:
Inoculum:
Incubation:
Reading:
Quality control:

EUCAST method for antimicrobial susceptibility testing by disk diffusion and recommendations for quality control

Breakpoints with a species name apply only to that particular species (in this example *S. aureus*)

Antimicrobial agent	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Antimicrobial agent A	1[1]	1[1]	X	20[A]	20[A]	**1.** Notes that are general comments and/or relating to MIC breakpoints.
Antimicrobial agent B, *S. aureus*	2[2]	4	Y	26	23	**2.** New comment ~~Removed comment~~
Antimicrobial agent C	IE	IE		IE	IE	
Antimicrobial agent D	-	-		-	-	**A**. Comment on disk diffusion
Antimicrobial agent E	IP	IP		IP	IP	
Antimicrobial agent F (screen)	NA	NA	Y	25	25	
Antimicrobial agent G	0.5	2	Z	30	24	

Changes from previous version highlighted in yellow

Screening breakpoint to differentiate between isolates without and with resistance mechanisms

Not Applicable

In Preparation

No breakpoints. Susceptibility testing is not recommended

MIC breakpoints in blue are linked to MIC distributions

Zone diameter breakpoints in blue are linked to zone diameter distributions

Antimicrobial agents in blue are linked to EUCAST rationale documents

Insufficient evidence that the organism or group is a good target for therapy with the agent

European Committee on Antimicrobial Susceptibility Testing

Breakpoint tables for interpretation of MICs and zone diameters

Version 7.1, valid from 2017-03-10

Version 7.1, 2017-03-10	**Changes (cells containing a change, a deletion or an addition) from v. 7.0 are marked light blue.** **Changed comments are underlined. Removed comments are shown in strikethrough font style.**
***Staphylococcus* spp.**	**Revised breakpoints** • Cefoxitin screen for *S. epidermidis* (zone diameter) • Cefoxitin screen for *S. pseudintermedius* removed and replaced with oxacillin (zone diameter). Cefoxitin MIC breakpoint changed from Note[4] to "NA". **New comments** • Cephalosporins comment E **Revised comments** • Cephalosporins comment B
Topical agents	• Mupirocin ECOFF changed from 1/1 to 1 mg/L (typo)
Dosages	• Amoxicillin-clavulanic acid standard and high dose revised • Ceftazidime-avibactam high dose removed (typo)

European Committee on Antimicrobial Susceptibility Testing

Breakpoint tables for interpretation of MICs and zone diameters

Version 7.1, valid from 2017-03-10

Version 7.0, 2017-01-01	**Changes (cells containing a change, a deletion or an addition) from v. 6.0 are marked yellow.** **Changed comments are underlined. Removed comments are shown in strikethrough font style.**
General	• Link to Guidance Document on how to test and interpret results when there are no breakpoints added to content table. • Link to ceftobiprole rational document added. • Temocillin, ceftazidime-avibactam and nitroxoline added. • Norfloxacin indication added to antimicrobial name column. • Mupirocin breakpoints moved to separate table for topical agents.
Enterobacteriaceae	**General** • Pictures with reading examples for the fosfomycin disk diffusion test added **New breakpoints** • Temocillin (information added, see note) • Ceftazidime-avibactam (MIC and zone diameter) • Fosfomycin iv and oral (zone diameter) • Nitroxoline (MIC and zone diameter) **Revised breakpoints** • Cefepime (zone diameter) • Ceftriaxone (zone diameter) • Cefuroxime iv and oral (zone diameter) • Aztreonam (zone diameter) • Ciprofloxacin (MIC and zone diameter) • Levofloxacin (MIC and zone diameter) • Moxifloxacin (MIC and zone diameter) • Norfloxacin (valid for uncomplicated UTI only) • Ofloxacin (MIC and zone diameter) • Trimethoprim-sulfamethoxazole (zone diameter) **New comments** • Penicillins comments 5 and 6 • Cephalosporins comment 3 • Miscellaneous agents comment 1 • Miscellaneous agents comments B, C and D **Revised comments** • Miscellaneous agents comment 2
***Pseudomonas* spp.**	**New breakpoints** • Ceftazidime-avibactam (MIC and zone diameter for *P. aeruginosa*) **Revised breakpoints** • Ciprofloxacin (MIC and zone diameter) • Levofloxacin (MIC and zone diameter) • Colistin (MIC) **New comments** • Cephalosporins comment 3 • Fluoroquinolones comments 1-2 • Miscellaneous agents comment 1
Stenotrophomonas maltophilia	**Revised comments** • Miscellaneous agents comment A

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Breakpoint tables for interpretation of MICs and zone diameters

Version 7.1, valid from 2017-03-10

***Acinetobacter* spp.**	**Revised breakpoints** • Doripenem (zone diameter) • Levofloxacin (MIC and zone diameter) • Amikacin (zone diameter) • Trimethoprim-sulfamethoxazole (zone diameter) **New comments** • Fluoroquinolones comment 1 • Miscellaneous agents comment 1
***Staphylococcus* spp.**	**Revised breakpoints** • Cefoxitin screen for coagulase-negative staphylococci (zone diameter) • Ciprofloxacin (zone diameter - separate breakpoints for *S. aureus* and coagulase-negative staphylococci) • Levofloxacin (MIC and zone diameter - separate breakpoints for *S. aureus* and coagulase-negative staphylococci) • Moxifloxacin (MIC and zone diameter - separate breakpoints for *S. aureus* and coagulase-negative staphylococci) • Ofloxacin (zone diameter - separate breakpoints for coagulase-negative staphylococci) • Linezolid (zone diameter) • Mupirocin (breakpoints moved to Topical agent table, where zone diameter breakpoints are available as a note) **New comments** • Cephalosporins comment B **Revised comments** • Penicillins comment B • Cephalosporins comments 1/A and 2 • Aminoglycosides comment 2 • Miscellaneous agents comment 4/B (comment moved to Topical agent table)
Streptococcus groups A, B, C and G	**Revised breakpoints** • Levofloxacin (MIC and zone diameter) • Moxifloxacin (MIC and zone diameter)
Streptococcus pneumoniae	**General** • Flow chart instead of supplementary table for screening for beta-lactam resistance (no changes in algorithm) **Revised breakpoints** • Levofloxacin (zone diameter) • Norfloxacin screen (zone diameter) **Removed breakpoints** • Ciprofloxacin (MIC and zone diameter breakpoints) • Ofloxacin (MIC and zone diameter breakpoints) **Revised comments** • Fluoroquinolones comment B **Removed comments** • Fluoroquinolones previous comments 1 and 3
Viridans group streptococci	**Revised breakpoints** • Levofloxacin (MIC and zone diameter, changed to IE) • Moxifloxacin (MIC and zone diameter, changed to IE)

European Committee on Antimicrobial Susceptibility Testing

Breakpoint tables for interpretation of MICs and zone diameters

Version 7.1, valid from 2017-03-10

Haemophilus influenzae	**General** • Piperacillin-tazobactam note B added (typo error in previous versions) • Flow chart instead of supplementary table for screening for beta-lactam resistance (no changes in algorithm) **Revised breakpoints** • Cefepime (zone diameter) • Cefixime (zone diameter) • Cefotaxime (zone diameter) • Ceftaroline (zone diameter, changed from IP to Note) • Ceftriaxone (zone diameter) • Ciprofloxacin (MIC and zone diameter) • Levofloxacin (MIC and zone diameter) • Moxifloxacin (MIC and zone diameter) • Ofloxacin (MIC and zone diameter) **New comments** • Penicillins comment 2 **Revised comments** • Penicillins comment 1 **Removed comments** • Fluoroquinolones previous comment 1
Neisseria gonorrhoeae	**General** • General information on susceptibility testing updated. **Revised comments** • Penicillins comment 1 **Removed comments** • Tetracyclines previous comment 1
Neisseria meningitidis	**General** • Indication for meropenem moved from comment to antimicrobial name.
Pasteurella multocida	**Revised breakpoints** • Amoxicillin (zone diameter) **Removed breakpoints** • Ampicillin (zone diameter) **Revised comments** • Penicillins comment A
Aerococcus sanguinicola* and *urinae	• New table
Kingella kingae	• New table
Topical agents	• Table moved from Guidance document to Breakpoint Table. Updated clinical breakpoints are highlighted in yellow.
PK/PD (Non-species related) breakpoints	**New breakpoints** • Ceftazidime-avibactam **Revised breakpoints** • Ciprofloxacin • Levofloxacin • Moxifloxacin • Norfloxacin • Ofloxacin
Dosages	• Several dosages added or revised. • Dosages of inhibitors added for beta-lactam beta-lactamase inhibitor combination agents.

Enterobacteriaceae

EUCAST Clinical Breakpoint Tables v. 7.1, valid from 2017-03-10

Disk diffusion (EUCAST standardised disk diffusion method)
Medium: Mueller-Hinton agar
Inoculum: McFarland 0.5
Incubation: Air, 35±1ºC, 18±2h
Reading: Read zone edges as the point showing no growth viewed from the back of the plate against a dark background illuminated with reflected light.
Quality control: *Escherichia coli* ATCC 25922. For control of the inhibitor component of beta-lactam inhibitor-combination disks, use either *Escherichia coli* ATCC 35218 or *Klebsiella pneumoniae* ATCC 700603.

Penicillins[1]	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)	
	S ≤	R >		S ≥	R <
Benzylpenicillin	-	-		-	-
Ampicillin	8[1]	8	10	14[A,B]	14[B]
Ampicillin-sulbactam	8[1,2]	8[2]	10-10	14[A,B]	14[B]
Amoxicillin	8[1]	8	-	Note[C]	Note[C]
Amoxicillin-clavulanic acid	8[1,3]	8[3]	20-10	19[A,B]	19[B]
Amoxicillin-clavulanic acid (uncomplicated UTI only)	32[1,3]	32[3]	20-10	16[A,B]	16[B]
Piperacillin	8	16	30	20	17
Piperacillin-tazobactam	8[4]	16[4]	30-6	20	17
Ticarcillin	8	16	75	23	23
Ticarcillin-clavulanic acid	8[3]	16[3]	75-10	23	23
Temocillin	Note[5]	Note[5]		Note[5]	Note[5]
Phenoxymethylpenicillin	-	-		-	-
Oxacillin	-	-		-	-
Cloxacillin	-	-		-	-
Dicloxacillin	-	-		-	-
Flucloxacillin	-	-		-	-
Mecillinam (uncomplicated UTI only) *E. coli, Klebsiella* spp. and *P. mirabilis*	8[6]	8[6]	10	15[D]	15[D]

Notes
Numbered notes relate to general comments and/or MIC breakpoints.
Lettered notes relate to the disk diffusion method.

1/A. Wild type Enterobacteriaceae are categorised as susceptible to aminopenicillins.
Some countries prefer to categorise wild type isolates of *E. coli* and *P. mirabilis* as intermediate. When this is the case, use the MIC breakpoint S ≤ 0.5 mg/L and the corresponding zone diameter breakpoint S ≥ 50 mm.
2. For susceptibility testing purposes, the concentration of sulbactam is fixed at 4 mg/L.
3. For susceptibility testing purposes, the concentration of clavulanic acid is fixed at 2 mg/L.
4. For susceptibility testing purposes, the concentration of tazobactam is fixed at 4 mg/L.
5. Breakpoints are pending applications to national regulatory authorities to license a high-dose regimen of 2 g x 3.
6. Agar dilution is the reference method for mecillinam MIC determination.

B. Ignore growth that may appear as a thin inner zone on some batches of Mueller-Hinton agars.
C. Susceptibility inferred from ampicillin.
D. Ignore isolated colonies within the inhibition zone for *E. coli.*

Cephalosporins[1]	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Cefaclor	-	-		-	-	**1.** The cephalosporin breakpoints for Enterobacteriaceae will detect all clinically important resistance mechanisms (including ESBL and plasmid mediated AmpC). Some isolates that produce beta-lactamases are susceptible or intermediate to 3rd or 4th generation cephalosporins with these breakpoints and should be reported as tested, *i.e.* the presence or absence of an ESBL does not in itself influence the categorisation of susceptibility. ESBL detection and characterisation are recommended for public health and infection control purposes.
Cefadroxil (uncomplicated UTI only)	16	16	**30**	12	12	
Cefalexin (uncomplicated UTI only)	16	16	**30**	14	14	
Cefazolin	-	-		-	-	
Cefepime	1	4	**30**	27	21	**2.** The cefoxitin ECOFF (8 mg/L) has a high sensitivity but poor specificity for identification of AmpC-producing Enterobacteriaceae as this agent is also affected by permeability alterations and some carbapenemases. Classical non-AmpC producers are wild type, whereas plasmid AmpC producers or chromosomal AmpC hyperproducers are non-wild type.
Cefixime (uncomplicated UTI only)	1	1	**5**	17	17	
Cefotaxime	1	2	**5**	20	17	
Cefoxitin (screen)[2]	NA	NA	**30**	19	19	**3.** For susceptibility testing purposes, the concentration of avibactam is fixed at 4 mg/L.
Cefpodoxime (uncomplicated UTI only)	1	1	**10**	21	21	**4.** For susceptibility testing purposes, the concentration of tazobactam is fixed at 4 mg/L.
Ceftaroline	0.5	0.5	**5**	23	23	**5.** Breakpoints are based on high dose therapy (1.5 g x 3).
Ceftazidime	1	4	**10**	22	19	
Ceftazidime-avibactam	8[3]	8[3]	**10-4**	13	13	
Ceftibuten (UTI only)	1	1	**30**	23	23	
Ceftobiprole	0.25	0.25	**5**	23	23	
Ceftolozane-tazobactam	1[4]	1[4]	**30-10**	23	23	
Ceftriaxone	1	2	**30**	25	22	
Cefuroxime iv[5], *E. coli, Klebsiella* spp. and *P. mirabilis*	8	8	**30**	19	19	
Cefuroxime oral (uncomplicated UTI only)	8	8	**30**	19	19	

Carbapenems[1]	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Doripenem	1	2	**10**	24	21	**1.** The carbapenem breakpoints for Enterobacteriaceae will detect all clinically important resistance mechanisms (including the majority of carbapenemases). Some isolates that produce carbapenemase are categorised as susceptible with these breakpoints and should be reported as tested, *i.e.* the presence or absence of a carbapenemase does not in itself influence the categorisation of susceptibility. Carbapenemase detection and characterisation are recommended for public health and infection control purposes.
Ertapenem	0.5	1	**10**	25	22	
Imipenem[2]	2	8	**10**	22	16	
Meropenem	2	8	**10**	22	16	**2.** Low-level resistance is common in *Morganella* spp., *Proteus* spp. and *Providencia* spp.

Monobactams	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Aztreonam[1]	1	4	**30**	26	21	**1.** The aztreonam breakpoints for Enterobacteriaceae will detect clinically important resistance mechanisms (including ESBL). Some isolates that produce beta-lactamases are susceptible or intermediate to aztreonam with these breakpoints and should be reported as tested, *i.e.* the presence or absence of an ESBL does not in itself influence the categorisation of susceptibility. ESBL detection and characterisation are recommended for public health and infection control purposes.

Fluoroquinolones	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Ciprofloxacin	0.25	0.5	**5**	26	24	**1**. There is clinical evidence for ciprofloxacin to indicate a poor response in systemic infections caused by *Salmonella* spp. with low-level ciprofloxacin resistance (MIC >0.06 mg/L). The available data relate mainly to *Salmonella* Typhi but there are also case reports of poor response with other *Salmonella* species.
Ciprofloxacin, *Salmonella* spp.[1]	0.06	0.06		Note[A]	Note[A]	
Pefloxacin (screen), *Salmonella* spp.[1]	NA	NA	**5**	24[B]	24[B]	
Levofloxacin	0.5	1	**5**	23	19	**A**. Tests with a ciprofloxacin 5 µg disk will not reliably detect low-level resistance in *Salmonella* spp. To screen for ciprofloxacin resistance in *Salmonella* spp., use the pefloxacin 5 µg disk. **See Note B.**
Moxifloxacin	0.25	0.25	**5**	22	22	
Nalidixic acid (screen)	NA	NA		NA	NA	**B.** Susceptibility of *Salmonella* spp. to ciprofloxacin can be inferred from pefloxacin disk diffusion susceptibility.
Norfloxacin (uncomplicated UTI only)	0.5	1	**10**	22	19	
Ofloxacin	0.25	0.5	**5**	24	22	

Aminoglycosides[1]	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Amikacin	8	16	**30**	18	15	**1.** Aminoglycoside breakpoints are based on once-daily administration of high aminoglycoside dosages. Most often aminoglycosides are given in combination with beta-lactam agents.
Gentamicin	2	4	**10**	17	14	
Netilmicin	2	4	**10**	15	12	
Tobramycin	2	4	**10**	17	14	

Glycopeptides and lipoglycopeptides	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R>		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Dalbavancin	-	-		-	-	
Oritavancin	-	-		-	-	
Teicoplanin	-	-		-	-	
Telavancin	-	-		-	-	
Vancomycin	-	-		-	-	

Macrolides, lincosamides and streptogramins	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
	S ≤	R >		S ≥	R <	
Azithromycin[1]	-	-		-	-	**1.** Azithromycin has been used in the treatment of infections with *Salmonella* Typhi (MIC ≤16 mg/L for wild type isolates) and *Shigella* spp.
Clarithromycin	-	-		-	-	
Erythromycin	-	-		-	-	
Roxithromycin	-	-		-	-	
Telithromycin	-	-		-	-	
Clindamycin	-	-		-	-	
Quinupristin-dalfopristin	-	-		-	-	

Tetracyclines	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
	S ≤	R >		S ≥	R <	
Doxycycline	-	-		-	-	**1**. Tigecycline has poor activity against *Morganella* spp., *Proteus* spp. and *Providencia* spp.
Minocycline	-	-		-	-	**2.** For tigecycline broth microdilution MIC determination, the medium must be prepared fresh on the day of use.
Tetracycline	-	-		-	-	
Tigecycline[1]	1[2]	2[2]	15	18[A]	15[A]	**A.** Zone diameter breakpoints validated for *E. coli* only. For other Enterobacteriaceae, use an MIC method.

Oxazolidinones	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
	S ≤	R >		S ≥	R <	
Linezolid	-	-		-	-	
Tedizolid	-	-		-	-	

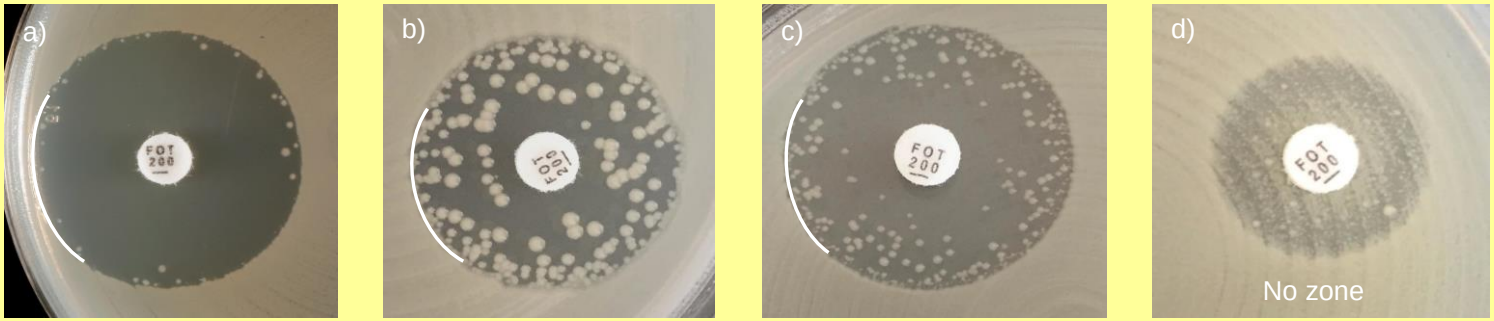
Miscellaneous agents	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)	
	S ≤	R >		S ≥	R <
Chloramphenicol	8	8	**30**	17	17
Colistin[1]	2	2		Note[A]	Note[A]
Daptomycin	-	-		-	-
Fosfomycin iv	32[2]	32[2]	**200**[B]	24[C,D]	24[C,D]
Fosfomycin oral (uncomplicated UTI only)	32[2]	32[2]	**200**[B]	24[C,D]	24[C,D]
Fusidic acid	-	-		-	-
Metronidazole	-	-		-	-
~~Mupirocin~~					
Nitrofurantoin (uncomplicated UTI only), *E. coli*	64	64	**100**	11	11
Nitroxoline (uncomplicated UTI only), *E. coli*	16	16	**30**	15	15
Rifampicin	-	-		-	-
Spectinomycin	-	-		-	-
Trimethoprim (uncomplicated UTI only)	2	4	**5**	18	15
Trimethoprim-sulfamethoxazole[3]	2	4	**1.25-23.75**	14	11

Notes

Numbered notes relate to general comments and/or MIC breakpoints.
Lettered notes relate to the disk diffusion method.

1. Quality control of colistin must be performed with both a susceptible QC strain (*E. coli* ATCC 25922 or *P. aeruginosa* ATCC 27853) and the colistin resistant *E. coli* NCTC 13846 (*mcr-1* positive).
2. Agar dilution is the reference method for fosfomycin. MICs must be determined in the presence of glucose-6-phosphate (25 mg/L in the medium). Follow the manufacturers' instructions for commercial systems.
3. Trimethoprim:sulfamethoxazole in the ratio 1:19. Breakpoints are expressed as the trimethoprim concentration.

A. Use an MIC method.
B. Fosfomycin 200 µg disks must contain 50 µg glucose-6-phosphate.
C. Zone diameter breakpoints apply to *E. coli* only. For other Enterobacteriaceae, use an MIC method.
D. Ignore isolated colonies within the inhibition zone (**see pictures below**).



Examples of inhibition zones for *Escherichia coli* with fosfomycin.
a-c) Ignore all colonies and read the outer zone edge.
d) Record as no inhibition zone.

Disk diffusion (EUCAST standardised disk diffusion method)
Medium: Mueller-Hinton agar
Inoculum: McFarland 0.5
Incubation: Air, 35±1ºC, 18±2h
Reading: Read zone edges as the point showing no growth viewed from the back of the plate against a dark background illuminated with reflected light.
Quality control: *Pseudomonas aeruginosa* ATCC 27853. For control of the inhibitor component of beta-lactam inhibitor-combination disks, use either *Escherichia coli* ATCC 35218 or *Klebsiella pneumoniae* ATCC 700603.

Penicillins	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)	
	S ≤	R >		S ≥	R <
Benzylpenicillin	-	-		-	-
Ampicillin	-	-		-	-
Ampicillin-sulbactam	-	-		-	-
Amoxicillin	-	-		-	-
Amoxicillin-clavulanic acid	-	-		-	-
Piperacillin[1]	16	16	30	18	18
Piperacillin-tazobactam[1]	16[2]	16[2]	30-6	18	18
Ticarcillin[3]	16	16	75	18	18
Ticarcillin-clavulanic acid[3]	16[4]	16[4]	75-10	18	18
Temocillin	-	-		-	-
Phenoxymethylpenicillin	-	-		-	-
Oxacillin	-	-		-	-
Cloxacillin	-	-		-	-
Dicloxacillin	-	-		-	-
Flucloxacillin	-	-		-	-
Mecillinam (uncomplicated UTI only)	-	-		-	-

Notes
Numbered notes relate to general comments and/or MIC breakpoints.
Lettered notes relate to the disk diffusion method.

1. Breakpoints are based on high dose therapy (4 g x 4, with or without tazobactam).
2. For susceptibility testing purposes, the concentration of tazobactam is fixed at 4 mg/L.
3. Breakpoints are based on a dose of at least 3 g x 4, with or without clavulanic acid.
4. For susceptibility testing purposes, the concentration of clavulanic acid is fixed at 2 mg/L.

Cephalosporins	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Cefaclor	-	-		-	-	**1.** Breakpoints are based on high dose therapy (2 g x 3).
Cefadroxil	-	-		-	-	**2**. Breakpoints are based on high dose therapy (2 g x 3).
Cefalexin	-	-		-	-	**3.** For susceptibility testing purposes, the concentration of avibactam is fixed at 4 mg/L.
Cefazolin	-	-		-	-	**4.** For susceptibility testing purposes, the concentration of tazobactam is fixed at 4 mg/L.
Cefepime[1]	8	8	**30**	19	19	
Cefixime	-	-		-	-	
Cefotaxime	-	-		-	-	
Cefoxitin	NA	NA		NA	NA	
Cefpodoxime	-	-		-	-	
Ceftaroline	-	-		-	-	
Ceftazidime[2]	8	8	**10**	17	17	
Ceftazidime-avibactam, *P. aeruginosa*	8[3]	8[3]	**10-4**	17	17	
Ceftibuten	-	-		-	-	
Ceftobiprole	IE	IE		IE	IE	
Ceftolozane-tazobactam, *P. aeruginosa*	4[4]	4[4]	**30-10**	IP	IP	
Ceftriaxone	-	-		-	-	
Cefuroxime iv	-	-		-	-	
Cefuroxime oral	-	-		-	-	

Carbapenems	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Doripenem[1]	1	2	**10**	25	22	**1.** Breakpoints are based on high dose therapy (1 g administered over 4 h x 3).
Ertapenem	-	-		-	-	**2.** Breakpoints are based on high dose therapy (1 g x 4).
Imipenem[2]	4	8	**10**	20	17	
Meropenem	2	8	**10**	24	18	

Monobactams	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Aztreonam	1	16	**30**	50	16	

Fluoroquinolones	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Ciprofloxacin[1]	0.5	0.5	5	26	26	1. Breakpoints are based on high dose therapy (0.75 g x 2 oral or 0.4 g x 3 iv).
Levofloxacin[2]	1	1	5	22	22	2. Breakpoints are based on high dose therapy (0.5 g x 2 oral or 0.5 g x 2 iv).
Moxifloxacin	-	-		-	-	
Nalidixic acid (screen)	NA	NA		NA	NA	
Norfloxacin (uncomplicated UTI only)	-	-		-	-	
Ofloxacin	-	-		-	-	

Aminoglycosides[1]	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Amikacin	8	16	30	18	15	**1**. Aminoglycoside breakpoints are based on once-daily administration of high aminoglycoside dosages. Most often aminoglycosides are given in combination with beta-lactam agents.
Gentamicin	4	4	10	15	15	
Netilmicin	4	4	10	12	12	
Tobramycin	4	4	10	16	16	

Glycopeptides and lipoglycopeptides	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Dalbavancin	-	-		-	-	
Oritavancin	-	-		-	-	
Teicoplanin	-	-		-	-	
Telavancin	-	-		-	-	
Vancomycin	-	-		-	-	

Macrolides, lincosamides and streptogramins	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Azithromycin	-	-		-	-	
Clarithromycin	-	-		-	-	
Erythromycin	-	-		-	-	
Roxithromycin	-	-		-	-	
Telithromycin	-	-		-	-	
Clindamycin	-	-		-	-	
Quinupristin-dalfopristin	-	-		-	-	

Tetracyclines	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Doxycycline	-	-		-	-	
Minocycline	-	-		-	-	
Tetracycline	-	-		-	-	
Tigecycline	-	-		-	-	

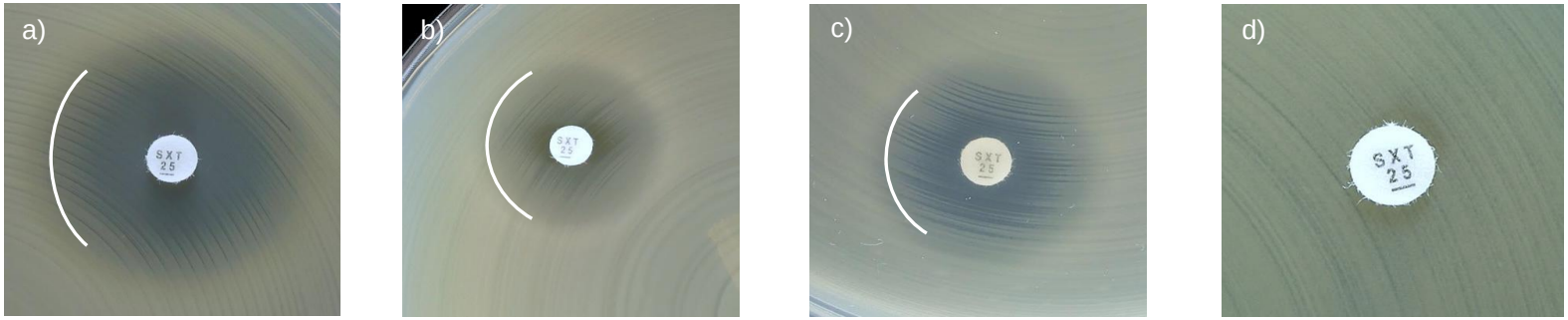
Oxazolidinones	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Linezolid	-	-		-	-	
Tedizolid	-	-		-	-	

Miscellaneous agents	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Chloramphenicol	-	-		-	-	**1.** Quality control of colistin must be performed with both a susceptible QC strain (*E. coli* ATCC 25922 or *P. aeruginosa* ATCC 27853) and the colistin resistant *E. coli* NCTC 13846 (*mcr-1* positive).
Colistin[1]	2	2		Note[A]	Note[A]	**2.** Infections caused by wild type isolates (ECOFF 128 mg/L) have been treated with combinations of fosfomycin and other agents.
Daptomycin	-	-		-	-	
Fosfomycin iv[2]	-	-		-	-	**A.** Use an MIC method.
Fosfomycin oral[2]	-	-		-	-	
Fusidic acid	-	-		-	-	
Metronidazole	-	-		-	-	
~~Mupirocin~~						
Nitrofurantoin (uncomplicated UTI only)	-	-		-	-	
Nitroxoline (uncomplicated UTI only)	-	-		-	-	
Rifampicin	-	-		-	-	
Spectinomycin	-	-		-	-	
Trimethoprim (uncomplicated UTI only)	-	-		-	-	
Trimethoprim-sulfamethoxazole	-	-		-	-	

Trimethoprim-sulfamethoxazole is the only agent for which EUCAST breakpoints are currently available. For further information, see guidance document on www.eucast.org.

Disk diffusion (EUCAST standardised disk diffusion method)
Medium: Mueller-Hinton agar
Inoculum: McFarland 0.5
Incubation: Air, 35±1ºC, 18±2h
Reading: Read zone edges from the back of the plate against a dark background illuminated with reflected light (see below for specific instructions).
Quality control: *Escherichia coli* ATCC 25922

Miscellaneous agents	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Trimethoprim-sulfamethoxazole[1,2]	4	4	**1.25-23.75**	16[A]	16[A]	**1.** Trimethoprim:sulfamethoxazole in the ratio 1:19. Breakpoints are expressed as the trimethoprim concentration. **2.** Breakpoints are based on high dose therapy, at least 0.24 g trimethoprim and 1.2 g sulfamethoxazole administered together twice daily. **A.** Isolates showing any sign of inhibition zone ≥ 16 mm should be reported susceptible and growth within the inhibition zone should be ignored. The density of growth within the zone may vary from a fine haze to substantial growth (**see pictures below**).



Examples of inhibition zones for *Stenotrophomonas maltophilia* with trimethoprim-sulfamethoxazole.
a-c) An outer zone can be seen. Report susceptible if the zone diameter ≥ 16 mm.
d) Growth up to the disk **and** no sign of inhibition zone. Report resistant.

Disk diffusion (EUCAST standardised disk diffusion method)
Medium: Mueller-Hinton agar
Inoculum: McFarland 0.5
Incubation: Air, 35±1ºC, 18±2h
Reading: Read zone edges as the point showing no growth viewed from the back of the plate against a dark background illuminated with reflected light.
Quality control: *Pseudomonas aeruginosa* ATCC 27853.

Penicillins[1]	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Benzylpenicillin	-	-		-	-	**1.** Susceptibility testing of *Acinetobacter* spp. to penicillins is unreliable. In most instances, *Acinetobacter* spp. are resistant to penicillins.
Ampicillin	-	-		-	-	
Ampicillin-sulbactam	IE	IE		IE	IE	
Amoxicillin	-	-		-	-	
Amoxicillin-clavulanic acid	-	-		-	-	
Piperacillin	IE	IE		IE	IE	
Piperacillin-tazobactam	IE	IE		IE	IE	
Ticarcillin	IE	IE		IE	IE	
Ticarcillin-clavulanic acid	IE	IE		IE	IE	
Temocillin	-	-		-	-	
Phenoxymethylpenicillin	-	-		-	-	
Oxacillin	-	-		-	-	
Cloxacillin	-	-		-	-	
Dicloxacillin	-	-		-	-	
Flucloxacillin	-	-		-	-	
Mecillinam (uncomplicated UTI only)	-	-		-	-	

Cephalosporins	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Cefaclor	-	-		-	-	
Cefadroxil	-	-		-	-	
Cefalexin	-	-		-	-	
Cefazolin	-	-		-	-	
Cefepime	-	-		-	-	
Cefixime	-	-		-	-	
Cefotaxime	-	-		-	-	
Cefoxitin	-	-		-	-	
Cefpodoxime	-	-		-	-	
Ceftaroline	-	-		-	-	
Ceftazidime	-	-		-	-	
Ceftazidime-avibactam	-	-		-	-	
Ceftibuten	-	-		-	-	
Ceftobiprole	-	-		-	-	
Ceftolozane-tazobactam	-	-		-	-	
Ceftriaxone	-	-		-	-	
Cefuroxime iv	-	-		-	-	
Cefuroxime oral	-	-		-	-	

Carbapenems	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Doripenem[1]	1	2	10	24	21	**1.** Breakpoints are based on high dose therapy (1 g administered over 4 h x 3).
Ertapenem	-	-		-	-	**2.** Breakpoints are based on high dose therapy (1 g x 4).
Imipenem[2]	2	8	10	23	17	
Meropenem	2	8	10	21	15	

Monobactams	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Aztreonam	-	-		-	-	

Fluoroquinolones	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Ciprofloxacin[1]	1	1	5	21	21	**1**. Breakpoints are based on high dose therapy (0.75 g x 2 oral or 0.4 g x 3 iv).
Levofloxacin	0.5	1	5	23	20	
Moxifloxacin	-	-		-	-	
Nalidixic acid (screen)	NA	NA		NA	NA	
Norfloxacin (uncomplicated UTI only)	-	-		-	-	
Ofloxacin	-	-		-	-	

Aminoglycosides[1]	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Amikacin	8	16	30	19	17	**1**. Aminoglycoside breakpoints are based on once-daily administration of high aminoglycoside dosages. Most often aminoglycosides are given in combination with beta-lactam agents.
Gentamicin	4	4	10	17	17	
Netilmicin	4	4	10	16	16	
Tobramycin	4	4	10	17	17	

Glycopeptides and lipoglycopeptides	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Dalbavancin	-	-		-	-	
Oritavancin	-	-		-	-	
Teicoplanin	-	-		-	-	
Telavancin	-	-		-	-	
Vancomycin	-	-		-	-	

Macrolides, lincosamides and streptogramins	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Azithromycin	-	-		-	-	
Clarithromycin	-	-		-	-	
Erythromycin	-	-		-	-	
Roxithromycin	-	-		-	-	
Telithromycin	-	-		-	-	
Clindamycin	-	-		-	-	
Quinupristin-dalfopristin	-	-		-	-	

Tetracyclines	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Doxycycline	-	-		-	-	
Minocycline	IE	IE		IE	IE	
Tetracycline	-	-		-	-	
Tigecycline	IE	IE		IE	IE	

Oxazolidinones	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Linezolid	-	-		-	-	
Tedizolid	-	-		-	-	

Miscellaneous agents	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Chloramphenicol	-	-		-	-	**1.** Quality control of colistin must be performed with both a susceptible QC strain (*E. coli* ATCC 25922 or *P. aeruginosa* ATCC 27853) and the colistin resistant *E. coli* NCTC 13846 (*mcr-1* positive).
Colistin[1]	2	2		Note[A]	Note[A]	**2.** Trimethoprim:sulfamethoxazole in the ratio 1:19. Breakpoints are expressed as the trimethoprim concentration.
Daptomycin	-	-		-	-	
Fosfomycin iv	-	-		-	-	**A.** Use an MIC method.
Fosfomycin oral	-	-		-	-	
Fusidic acid	-	-		-	-	
Metronidazole	-	-		-	-	
~~Mupirocin~~						
Nitrofurantoin (uncomplicated UTI only)	-	-		-	-	
Nitroxoline (uncomplicated UTI only)	-	-		-	-	
Rifampicin	-	-		-	-	
Spectinomycin	-	-		-	-	
Trimethoprim (uncomplicated UTI only)	-	-		-	-	
Trimethoprim-sulfamethoxazole[2]	2	4	1.25-23.75	14	11	

Staphylococcus spp.

Disk diffusion (EUCAST standardised disk diffusion method)
Medium: Mueller-Hinton agar
Inoculum: McFarland 0.5
Incubation: Air, 35±1ºC, 18±2h
Reading: Read zone edges as the point showing no growth viewed from the back of the plate against a dark background illuminated with reflected light (except for benzylpenicillin and linezolid, see below).
Quality control: *Staphylococcus aureus* ATCC 29213

Penicillins[1]	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)	
	S ≤	R >		S ≥	R <
Benzylpenicillin, *S. aureus*	0.125[1]	0.125[1]	**1 unit**	26[A,B]	26[A,B]
Benzylpenicillin, *S. lugdunensis*	0.125[1]	0.125[1]	**1 unit**	26[A]	26[A]
Benzylpenicillin, Coagulase-negative staphylococci	-[2]	-[2]		Note[C]	Note[C]
Ampicillin, *S. saprophyticus*	Note[1,3]	Note[1,3]	**2**	18[A,D]	18[A,D]
Ampicillin-sulbactam	Note[1,3]	Note[1,3]		Note[A,D]	Note[A,D]
Amoxicillin	Note[1,3]	Note[1,3]		Note[A,D]	Note[A,D]
Amoxicillin-clavulanic acid	Note[1,3]	Note[1,3]		Note[A,D]	Note[A,D]
Piperacillin	Note[1,3]	Note[1,3]		Note[A,D]	Note[A,D]
Piperacillin-tazobactam	Note[1,3]	Note[1,3]		Note[A,D]	Note[A,D]
Ticarcillin	Note[1]	Note[1]		Note[A]	Note[A]
Ticarcillin-clavulanic acid	Note[1]	Note[1]		Note[A]	Note[A]
Temocillin	-	-		-	-
Phenoxymethylpenicillin	Note[1]	Note[1]		Note[A]	Note[A]
Oxacillin[4]	Note[1,4]	Note[1,4]		Note[A]	Note[A]
Cloxacillin	Note[1]	Note[1]		Note[A]	Note[A]
Dicloxacillin	Note[1]	Note[1]		Note[A]	Note[A]
Flucloxacillin	Note[1]	Note[1]		Note[A]	Note[A]
Mecillinam (uncomplicated UTI only)	-	-		-	-

Notes

Numbered notes relate to general comments and/or MIC breakpoints.
Lettered notes relate to the disk diffusion method.

1/A. Most staphylococci are penicillinase producers, which are resistant to benzylpenicillin, phenoxymethylpenicillin, ampicillin, amoxicillin, piperacillin and ticarcillin. Isolates negative for penicillinase and susceptible to methicillin can be reported susceptible to these agents. Isolates positive for penicillinase and methicillin susceptible are susceptible to beta-lactamase inhibitor combinations and isoxazolylpenicillins (oxacillin, cloxacillin, dicloxacillin and flucloxacillin).
Methicillin resistant isolates are, with few exceptions, resistant to all beta-lactam agents.
2/C. No currently available method can reliably detect penicillinase production in coagulase-negative staphylococci.
3/D. Ampicillin susceptible *S. saprophyticus* are *mecA*-negative and susceptible to ampicillin, amoxicillin and piperacillin (without or with a beta-lactamase inhibitor).
4. *S. aureus, S. lugdunensis* and *S. saprophyticus* with oxacillin MIC values >2 mg/L are mostly methicillin resistant due to the presence of the *mecA* or *mecC* gene. The corresponding oxacillin MIC for coagulase-negative staphylococci other than *S. saprophyticus* and *S. lugdunensis* is >0.25 mg/L.

B. For *S. aureus*, disk diffusion is more reliable than MIC determination for detection of penicillinase producers, provided the zone diameter is measured AND the zone edge closely inspected (**see pictures below**). Examine the zone edge with transmitted light (plate held up to light). If the zone diameter is <26 mm, then report resistant. If the zone diameter is ≥26 mm AND the zone edge is sharp, then report resistant. If not sharp, then report susceptible and if uncertain, then report resistant. Chromogenic cephalosporin-based beta-lactamase tests do not reliably detect staphylococcal penicillinase.

Cephalosporins[1]	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Cefaclor[2]	Note[1]	Note[1]		Note[A]	Note[A]	**1/A.** Susceptibility of staphylococci to cephalosporins is inferred from the cefoxitin susceptibility except for cefixime, ceftazidime, ceftazidime-avibactam, ceftibuten and ceftolozane-tazobactam, which do not have breakpoints and should not be used for staphylococcal infections. Some methicillin-resistant *S. aureus* are susceptible to ceftaroline and ceftobiprole**, see Notes 5/C and 6/D.**
Cefadroxil	Note[1]	Note[1]		Note[A]	Note[A]	**2.** Breakpoints are based on a minimum dose of 500 mg x 3.
Cefalexin	Note[1]	Note[1]		Note[A]	Note[A]	**3.** *S. aureus* and *S. lugdunensis* with cefoxitin MIC values >4 mg/L and *S. saprophyticus* with cefoxitin MIC values >8 mg/L are methicillin resistant, mostly due to the presence of the *mecA* or *mecC* gene. Disk diffusion reliably predicts methicillin resistance.
Cefazolin	Note[1]	Note[1]		Note[A]	Note[A]	**4.** For staphylococci other than *S. aureus, S. lugdunensis* and *S. saprophyticus* , the cefoxitin MIC is a poorer predictor of methicillin resistance than the disk diffusion test.
Cefepime	Note[1]	Note[1]		Note[A]	Note[A]	**5/C.** Methicillin-susceptible isolates can be reported susceptible to ceftaroline without further testing.
Cefixime	-	-		-	-	**6/D.** Methicillin-susceptible isolates can be reported susceptible to ceftobiprole without further testing.
Cefotaxime	Note[1]	Note[1]		Note[A]	Note[A]	
Cefoxitin (screen), *S. aureus* and coagulase-negative staphylococci other than *S. epidermidis*	Note[3,4]	Note[3,4]	**30**	22[A,B]	22[A,B]	**B.** If coagulase-negative staphylococci are not identified to species level use zone diameter breakpoints S≥25, R<25 mm.
Cefoxitin (screen), *S. epidermidis*	Note[4]	Note[4]	**30**	25[A,B]	25[A,B]	**E.** Cefoxitin screen for methicillin resistance in *S. pseudintermedius* is less predictive of the presence of *mecA* than in other staphylococci. Use the oxacillin 1 µg disk with zone diameter breakpoints S≥20, R<20 mm to screen for methicillin resistance.
Cefoxitin (screen), *S. pseudintermedius*	NA	NA	**30**	Note[E]	Note[E]	
Cefpodoxime	Note[1]	Note[1]		Note[A]	Note[A]	
Ceftaroline, *S. aureus*	1[5]	1[5]	**5**	20[C]	20[C]	
Ceftazidime	-	-		-	-	
Ceftazidime-avibactam	-	-		-	-	
Ceftibuten	-	-		-	-	
Ceftobiprole, *S. aureus*	2[6]	2[6]	**5**	17[D]	17[D]	
Ceftolozane-tazobactam	-	-		-	-	
Ceftriaxone	Note[1]	Note[1]		Note[A]	Note[A]	
Cefuroxime iv	Note[1]	Note[1]		Note[A]	Note[A]	
Cefuroxime oral	Note[1]	Note[1]		Note[A]	Note[A]	

Carbapenems[1]	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Doripenem	Note[1]	Note[1]		Note[A]	Note[A]	1/A. Susceptibility of staphylococci to carbapenems is inferred from the cefoxitin susceptibility.
Ertapenem	Note[1]	Note[1]		Note[A]	Note[A]	
Imipenem	Note[1]	Note[1]		Note[A]	Note[A]	
Meropenem	Note[1]	Note[1]		Note[A]	Note[A]	

Monobactams	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Aztreonam	-	-		-	-	

Fluoroquinolones[1]	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
	S ≤	R >		S ≥	R <	
Ciprofloxacin[2], *S. aureus*	1	1	**5**	21[A]	21[A]	**1.** For breakpoints for other fluoroquinolones (*e.g.* pefloxacin and enoxacin), refer to breakpoints set by national breakpoint committees.
Ciprofloxacin[2], Coagulase-negative staphylococci	1	1	**5**	24[A]	24[A]	**2.** Breakpoints are based on high dose therapy (oral dose of 0.75 g x 2, iv dose of 0.4 g x 3).
Levofloxacin, *S. aureus*	1	1	**5**	22[A]	22[A]	**3.** Breakpoints are based on high dose therapy (0.4 g x 2).
Levofloxacin, Coagulase-negative staphylococci	1	1	**5**	24[A]	24[A]	
Moxifloxacin, *S. aureus*	0.25	0.25	**5**	25[A]	25[A]	**A.** The norfloxacin disk diffusion test can be used to screen for fluoroquinolone resistance. **See Note B.**
Moxifloxacin, Coagulase-negative staphylococci	0.25	0.25	**5**	28[A]	28[A]	**B.** Isolates categorised as susceptible to norfloxacin can be reported susceptible to ciprofloxacin, levofloxacin, moxifloxacin and ofloxacin. Isolates categorised as non-susceptible should be tested for susceptibility to individual agents.
Nalidixic acid (screen)	NA	NA		NA	NA	
Norfloxacin (screen)	NA	NA	**10**	17[B]	Note[B]	
Ofloxacin[3], *S. aureus*	1	1	**5**	20[A]	20[A]	
Ofloxacin[3], Coagulase-negative staphylococci	1	1	**5**	24[A]	24[A]	

Aminoglycosides[1]	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
	S ≤	R >		S ≥	R <	
Amikacin[2], *S. aureus*	8	16	**30**	18	16	**1.** Aminoglycoside breakpoints are based on once-daily administration.
Amikacin[2], Coagulase-negative staphylococci	8	16	**30**	22	19	**2.** Resistance to amikacin is most reliably determined by testing with kanamycin (MIC >8 mg/L). The corresponding zone diameter for the kanamyicn 30 µg disk is R<18 mm for *S. aureus* and R<22 mm for coagulase-negative staphylococci.
Gentamicin, *S. aureus*	1	1	**10**	18	18	
Gentamicin, Coagulase-negative staphylococci	1	1	**10**	22	22	
Netilmicin, *S. aureus*	1	1	**10**	18	18	
Netilmicin, Coagulase-negative staphylococci	1	1	**10**	22	22	
Tobramycin, *S. aureus*	1	1	**10**	18	18	
Tobramycin, Coagulase-negative staphylococci	1	1	**10**	22	22	

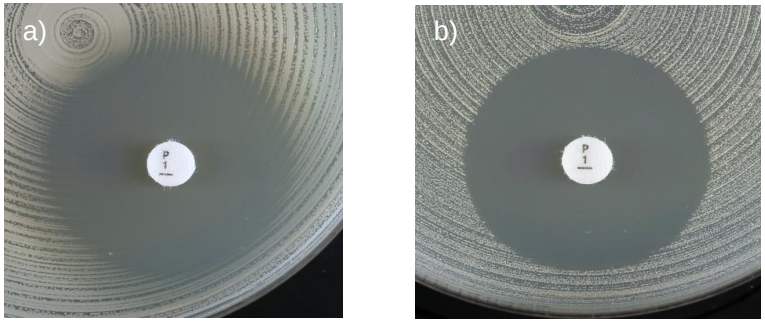
Glycopeptides and lipoglycopeptides[1]	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Dalbavancin[2]	0.125[3,4]	0.125[3]		Note[A]	Note[A]	**1.** Glycopeptide MICs are method dependent and should be determined by broth microdilution (reference ISO 20776). *S. aureus* with vancomycin MIC values of 2 mg/L are on the border of the wild type distribution and there may be an impaired clinical response. The resistant breakpoint has been reduced to 2 mg/L to avoid reporting "GISA" isolates intermediate as serious infections with "GISA" isolates are not treatable with increased doses of vancomycin or teicoplanin.
Oritavancin, *S. aureus*[2]	0.125[3,4]	0.125[3]		Note[A]	Note[A]	
Teicoplanin, *S. aureus*[2]	2	2		Note[A]	Note[A]	
Teicoplanin, Coagulase-negative staphylococci[2]	4	4		Note[A]	Note[A]	
Telavancin, MRSA[2]	0.125[3,5]	0.125[3]		Note[A]	Note[A]	**2**. Non-susceptible isolates are rare or not yet reported. The identification and antimicrobial susceptibility test result on any such isolate must be confirmed and the isolate sent to a reference laboratory.
Vancomycin, *S. aureus*[2]	2	2		Note[A]	Note[A]	**3.** MICs must be determined in the presence of polysorbate-80 (0.002% in the medium for broth dilution methods; agar dilution methods have not been validated). Follow the manufacturer's instructions for commercial systems.
Vancomycin, Coagulase-negative staphylococci[2]	4	4		Note[A]	Note[A]	**4.** *S. aureus* isolates susceptible to vancomycin can be reported susceptible to dalbavancin and oritavancin.
						5. MRSA isolates susceptible to vancomycin can be reported susceptible to telavancin.
						A. Disk diffusion is unreliable and cannot distinguish between wild type isolates and those with non-*vanA* -mediated glycopeptide resistance.

Macrolides, lincosamides and streptogramins	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Azithromycin	1[1]	2[1]		Note[A]	Note[A]	**1/A.** Erythromycin can be used to determine susceptibility to azithromycin, clarithromycin and roxithromycin.
Clarithromycin	1[1]	2[1]		Note[A]	Note[A]	**2.** Inducible clindamycin resistance can be detected by antagonism of clindamycin activity by a macrolide agent. If not detected, then report as susceptible. If detected, then report as resistant and consider adding this comment to the report: "Clindamycin may still be used for short-term therapy of less serious skin and soft tissue infections as constitutive resistance is unlikely to develop during such therapy".
Erythromycin	1[1]	2[1]	**15**	21[A]	18[A]	
Roxithromycin	1[1]	2[1]		Note[A]	Note[A]	
Telithromycin	IE	IE		IE	IE	
						B. Place the erythromycin and clindamycin disks 12-20 mm apart (edge to edge) and look for antagonism (the D phenomenon) to detect inducible clindamycin resistance.
Clindamycin[2]	0.25	0.5	**2**	22[B]	19[B]	**C.** Isolates non-susceptible by disk diffusion should be confirmed by MIC testing.
Quinupristin-dalfopristin	1	2	**15**	21	18[C]	

Tetracyclines	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Doxycycline	1[1]	2[1]		Note[A]	Note[A]	**1/A**. Isolates susceptible to tetracycline are also susceptible to doxycycline and minocycline, but some resistant to tetracycline may be susceptible to minocycline and/or doxycycline. An MIC method should be used to test doxycycline susceptibility of tetracycline resistant isolates if required.
Minocycline	0.5[1]	1[1]	**30**	23[A]	20[A]	
Tetracycline	1[1]	2[1]	**30**	22[A]	19[A]	
Tigecycline[2]	0.5[3]	0.5[3]	**15**	18	18	**2.** Non-susceptible isolates are rare or not yet reported. The identification and antimicrobial susceptibility test result on any such isolate must be confirmed and the isolate sent to a reference laboratory.
						3. For tigecycline broth microdilution MIC determination, the medium must be prepared fresh on the day of use.

Oxazolidinones	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Linezolid	4	4	10	21[A]	21[A]	**1.** Isolates susceptible to linezolid can be reported susceptible to tedizolid.
Tedizolid	0.5[1]	0.5		Note[B]	Note[B]	**A.** Examine zone edges with transmitted light (plate held up to light). **B.** Isolates susceptible to linezolid can be reported susceptible to tedizolid. For isolates resistant to linezolid, perform an MIC test.

Miscellaneous agents	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Chloramphenicol	8	8	30	18	18	**1.** Non-susceptible isolates are rare or not yet reported. The identification and antimicrobial susceptibility test result on any such isolate must be confirmed and the isolate sent to a reference laboratory.
Colistin	-	-		-	-	**2.** Daptomycin MICs must be determined in the presence of Ca^{2+} (50 mg/L in the medium for broth dilution methods; agar dilution methods have not been validated). Follow the manufacturers' instructions for commercial systems.
Daptomycin[1]	1[2]	1[2]		Note[A]	Note[A]	**3.** Fosfomycin MICs must be determined in the presence of glucose-6-phosphate (25 mg/L in the medium for broth and agar dilution methods). Follow the manufacturer's instructions for commercial systems.
Fosfomycin iv	32[3]	32[3]		Note[A]	Note[A]	**4/B.** ~~Breakpoints relate to nasal decolonisation of *S. aureus*. Intermediate isolates are associated with short term suppression (useful preoperatively) but, unlike susceptible isolates, long term eradication rates are low.~~
Fosfomycin oral	-	-		-	-	**4.** Trimethoprim:sulfamethoxazole in the ratio 1:19. Breakpoints are expressed as the trimethoprim concentration.
Fusidic acid	1	1	10	24	24	**A.** Use an MIC method.
Metronidazole	-	-		-	-	
~~Mupirocin~~						
Nitrofurantoin (uncomplicated UTI only), *S. saprophyticus*	64	64	100	13	13	
Nitroxoline (uncomplicated UTI only), *S. saprophyticus*	IE	IE		IE	IE	
Rifampicin	0.06	0.5	5	26	23	
Spectinomycin	-	-		-	-	
Trimethoprim (uncomplicated UTI only)	2	4	5	17	14	
Trimethoprim-sulfamethoxazole[4]	2	4	1.25-23.75	17	14	



Examples of inhibition zones for *Staphylococcus aureus* with benzylpenicillin.
a) Fuzzy zone edge and zone diameter ≥ 26 mm. Report susceptible.
b) Sharp zone edge and zone diameter ≥ 26 mm. Report resistant.

In endocarditis, refer to national or international endocarditis guidelines for breakpoints for *Enterococcus* spp.

Disk diffusion (EUCAST standardised disk diffusion method)
Medium: Mueller-Hinton agar
Inoculum: McFarland 0.5
Incubation: Air, 35±1ºC, 18±2h (for glycopeptides 24h)
Reading: Read zone edges as the point showing no growth viewed from the back of the plate against a dark background illuminated with reflected light (except for vancomycin, see below).
Quality control: *Enterococcus faecalis* ATCC 29212

Penicillins[1]	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Benzylpenicillin	-	-		-	-	**1.** *E. faecium* resistant to penicillins can be considered resistant to all other beta-lactam agents including carbapenems.
Ampicillin	4	8	2	10	8	**2/A.** Susceptibility to ampicillin, amoxicillin and piperacillin with and without beta-lactamase inhibitor can be inferred from ampicillin.
Ampicillin-sulbactam[2]	4[3]	8[3]		Note[A]	Note[A]	**3.** For susceptibility testing purposes, the concentration of sulbactam is fixed at 4 mg/L.
Amoxicillin[2]	4	8		Note[A]	Note[A]	**4.** For susceptibility testing purposes, the concentration of clavulanic acid is fixed at 2 mg/L.
Amoxicillin-clavulanic acid[2]	4[4]	8[4]		Note[A]	Note[A]	
Piperacillin[2]	Note[2]	Note[2]		Note[A]	Note[A]	
Piperacillin-tazobactam[2]	Note[2]	Note[2]		Note[A]	Note[A]	
Ticarcillin	-	-		-	-	
Ticarcillin-clavulanic acid	-	-		-	-	
Temocillin	-	-		-	-	
Phenoxymethylpenicillin	-	-		-	-	
Oxacillin	-	-		-	-	
Cloxacillin	-	-		-	-	
Dicloxacillin	-	-		-	-	
Flucloxacillin	-	-		-	-	
Mecillinam (uncomplicated UTI only)	-	-		-	-	

Cephalosporins	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Cefaclor	-	-		-	-	
Cefadroxil	-	-		-	-	
Cefalexin	-	-		-	-	
Cefazolin	-	-		-	-	
Cefepime	-	-		-	-	
Cefixime	-	-		-	-	
Cefotaxime	-	-		-	-	
Cefoxitin	-	-		-	-	
Cefpodoxime	-	-		-	-	
Ceftaroline	-	-		-	-	
Ceftazidime	-	-		-	-	
Ceftazidime-avibactam	-	-		-	-	
Ceftibuten	-	-		-	-	
Ceftobiprole	-	-		-	-	
Ceftolozane-tazobactam	-	-		-	-	
Ceftriaxone	-	-		-	-	
Cefuroxime iv	-	-		-	-	
Cefuroxime oral	-	-		-	-	

Carbapenems	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Doripenem	-	-		-	-	
Ertapenem	-	-		-	-	
Imipenem	4	8	10	21	18	
Meropenem	-	-		-	-	

Monobactams	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Aztreonam	-	-		-	-	

Fluoroquinolones	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Ciprofloxacin (uncomplicated UTI only)	4	4	5	15[A]	15[A]	**A.** The norfloxacin disk diffusion test can be used to screen for fluoroquinolone resistance. **See Note B.**
Levofloxacin (uncomplicated UTI only)	4	4	5	15[A]	15[A]	**B.** Susceptibility of ciprofloxacin and levofloxacin can be inferred from the norfloxacin susceptibility.
Moxifloxacin	-	-		-	-	
Nalidixic acid (screen)	NA	NA		NA	NA	
Norfloxacin (screen)	NA	NA	10	12[B]	12[B]	
Ofloxacin	-	-		-	-	

Aminoglycosides[1]	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Amikacin	Note[2]	Note[2]		Note[A]	Note[A]	**1**. Enterococci are intrinsically resistant to aminoglycosides and aminoglycoside monotherapy is ineffective. There is likely to be synergy between aminoglycosides and penicillins or glycopeptides against enterococci without acquired high-level resistance. All testing is therefore to distinguish between intrinsic and high-level acquired resistance.
Gentamicin (test for high-level aminoglycoside resistance)	Note[2]	Note[2]	30	Note[A]	Note[A]	**2/A.** Gentamicin can be used to screen for high-level aminoglycoside resistance (HLAR). **Negative test:** Isolates with gentamicin MIC ≤128 mg/L or a zone diameter ≥8 mm. The isolate is wild type for gentamicin and low-level intrinsic resistant. For other aminoglycosides, this may not be the case. Synergy with penicillins or glycopeptides can be expected if the isolate is susceptible to the penicillin or glycopeptide. **Positive test:** Isolates with gentamicin MIC >128 mg/L or a zone diameter <8 mm. The isolate is high-level resistant to gentamicin and other aminoglycosides, except streptomycin which must be tested separately if required (**see note 3/B**). There will be no synergy with penicillins or glycopeptides.
Netilmicin	Note[2]	Note[2]		Note[A]	Note[A]	**3/B.** Isolates with high-level gentamicin resistance may not be high-level resistant to streptomycin. **Negative test:** Isolates with streptomycin MIC ≤512 mg/L or a zone diameter ≥14 mm. The isolate is wild type for streptomycin and low-level intrinsic resistant. Synergy with penicillins or glycopeptides can be expected if the isolate is susceptible to the penicillin or glycopeptide. **Positive test:** Isolates with streptomycin MIC >512 mg/L or a zone diameter <14 mm. The isolate is high-level resistant to streptomycin. There will be no synergy with penicillins or glycopeptides.
Streptomycin (test for high-level streptomycin resistance)	Note[3]	Note[3]	300	Note[B]	Note[B]	
Tobramycin	Note[2]	Note[2]		Note[A]	Note[A]	

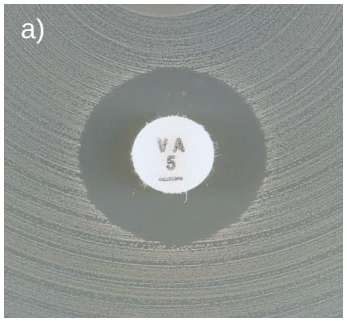
Glycopeptides and lipoglycopeptides	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Dalbavancin	IE	IE		IE	IE	**A.** Vancomycin susceptible enterococci exhibit sharp zone edges and do not exhibit colonies in the inhibition zone. Examine zone edges with transmitted light (plate held up to light). If the zone edge is fuzzy, colonies grow within the zone or if you are uncertain, then perform confirmatory testing with PCR or report resistant (**see pictures below**) even if the zone diameter is ≥ 12 mm. Isolates must not be reported susceptible before 24 h incubation.
Oritavancin	IE	IE		IE	IE	
Teicoplanin	2	2	30	16	16	
Telavancin	IE	IE		IE	IE	
Vancomycin	4	4	5	12[A]	12[A]	

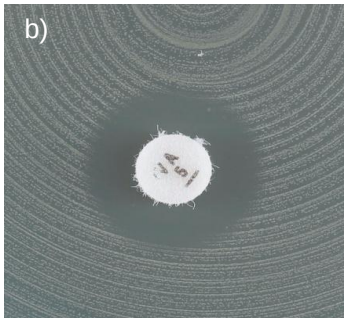
Macrolides, lincosamides and streptogramins	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Azithromycin	-	-		-	-	
Clarithromycin	-	-		-	-	
Erythromycin	-	-		-	-	
Roxithromycin	-	-		-	-	
Telithromycin	-	-		-	-	
				-	-	
Clindamycin	-	-		-	-	
Quinupristin-dalfopristin, *E. faecium*	1	4	**15**	22	20	

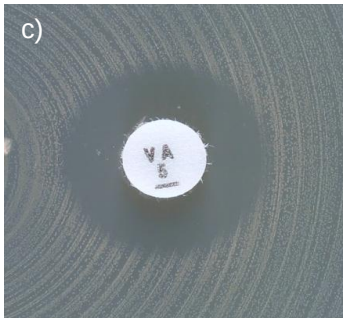
Tetracyclines	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Doxycycline	**-**	-		-	-	**1.** Non-susceptible isolates are rare or not yet reported. The identification and antimicrobial susceptibility test result on any such isolate must be confirmed and the isolate sent to a reference laboratory. **2** For tigecycline broth microdilution MIC determination, the medium must be prepared fresh on the day of use.
Minocycline	**-**	-		-	-	
Tetracycline	**-**	-		-	-	
Tigecycline[1]	0.25[2]	0.5[2]	**15**	18	15	

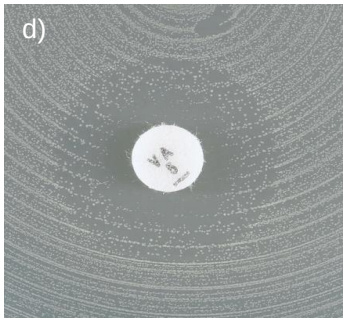
Oxazolidinones	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Linezolid	4	4	**10**	19	19	
Tedizolid	IE	IE		IE	IE	

Miscellaneous agents	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Chloramphenicol	-	-		-	-	**1.** For more information, see http://www.eucast.org/guidance_documents/.
Colistin	-	-		-	-	**2/A.** The activity of trimethoprim and trimethoprim-sulfamethozaxole is uncertain against enterococci, hence the wild type population is categorised as intermediate.
Daptomycin[1]	IE	IE		IE	IE	**3.** Trimethoprim-sulfamethoxazole in the ratio 1:19. Breakpoints are expressed as the trimethoprim concentration.
Fosfomycin iv	-	-		-	-	
Fosfomycin oral	-	-		-	-	
Fusidic acid	-	-		-	-	
Metronidazole	-	-		-	-	
~~**Mupirocin**~~						
Nitrofurantoin (uncomplicated UTI only), *E. faecalis*	64	64	**100**	15	15	
Nitroxoline (uncomplicated UTI only)	IE	IE		IE	IE	
Rifampicin	-	-		-	-	
Spectinomycin	-	-		-	-	
Trimethoprim (uncomplicated UTI only)	0.03[2]	1	**5**	50[A]	21	
Trimethoprim-sulfamethoxazole[3]	0.03[2]	1	**1.25-23.75**	50[A]	21	









Examples of inhibition zones for *Enterococcus* spp. with vancomycin.
a) Sharp zone edge **and** zone diameter ≥ 12 mm. Report susceptible.
b-d) Fuzzy zone edge or colonies within zone. Perform confirmatory testing with PCR or report resistant even if the zone diameter ≥ 12 mm.

Disk diffusion (EUCAST standardised disk diffusion method)
Medium: Mueller-Hinton agar + 5% defibrinated horse blood and 20 mg/L β-NAD (MH-F)
Inoculum: McFarland 0.5
Incubation: 5% CO_2, 35±1ºC, 18±2h
Reading: Read zone edges as the point showing no growth viewed from the front of the plate with the lid removed and with reflected light.
Quality control: *Streptococcus pneumoniae* ATCC 49619

Penicillins[1]	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Benzylpenicillin[2]	0.25	0.25	1 unit	18	18	**1/A**. The susceptibility of streptococcus groups A, B, C and G to penicillins is inferred from the benzylpenicillin susceptibility with the exception of phenoxymethylpenicillin and isoxazolylpenicillins for streptococcus group B.
Ampicillin	Note[1]	Note[1]		Note[A]	Note[A]	**2.** Non-susceptible isolates are rare or not yet reported. The identification and antimicrobial susceptibility test result on any such isolate must be confirmed and the isolate sent to a reference laboratory.
Ampicillin-sulbactam[3]	Note[1]	Note[1]		Note[A]	Note[A]	**3.** Streptococcus groups A, B, C and G do not produce beta-lactamase. The addition of a beta-lactamase inhibitor does not add clinical benefit.
Amoxicillin	Note[1]	Note[1]		Note[A]	Note[A]	
Amoxicillin-clavulanic acid[3]	Note[1]	Note[1]		Note[A]	Note[A]	
Piperacillin	Note[1]	Note[1]		Note[A]	Note[A]	
Piperacillin-tazobactam[3]	Note[1]	Note[1]		Note[A]	Note[A]	
Ticarcillin	-	-		-	-	
Ticarcillin-clavulanic acid	-	-		-	-	
Temocillin	-	-		-	-	
Phenoxymethylpenicillin Streptococcus groups A, C and G	Note[1]	Note[1]		Note[A]	Note[A]	
Oxacillin Streptococcus groups A, C and G	NA	NA		NA	NA	
Cloxacillin Streptococcus groups A, C and G	Note[1]	Note[1]		Note[A]	Note[A]	
Dicloxacillin Streptococcus groups A, C and G	Note[1]	Note[1]		Note[A]	Note[A]	
Flucloxacillin Streptococcus groups A, C and G	Note[1]	Note[1]		Note[A]	Note[A]	
Mecillinam (uncomplicated UTI only)	-	-		-	-	

Cephalosporins[1]	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Cefaclor	Note[1]	Note[1]		Note[A]	Note[A]	**1/A**. The susceptibility of streptococcus groups A, B, C and G to cephalosporins is inferred from the benzylpenicillin susceptibility.
Cefadroxil	Note[1]	Note[1]		Note[A]	Note[A]	
Cefalexin	Note[1]	Note[1]		Note[A]	Note[A]	
Cefazolin	Note[1]	Note[1]		Note[A]	Note[A]	
Cefepime	Note[1]	Note[1]		Note[A]	Note[A]	
Cefixime	-	-		-	-	
Cefotaxime	Note[1]	Note[1]		Note[A]	Note[A]	
Cefoxitin	NA	NA		NA	NA	
Cefpodoxime	Note[1]	Note[1]		Note[A]	Note[A]	
Ceftaroline	Note[1]	Note[1]		Note[A]	Note[A]	
Ceftazidime	-	-		-	-	
Ceftazidime-avibactam	-	-		-	-	
Ceftibuten	Note[1]	Note[1]		Note[A]	Note[A]	
Ceftobiprole	IE	IE		IE	IE	
Ceftolozane-tazobactam	IE	IE		IE	IE	
Ceftriaxone	Note[1]	Note[1]		Note[A]	Note[A]	
Cefuroxime iv	Note[1]	Note[1]		Note[A]	Note[A]	
Cefuroxime oral	Note[1]	Note[1]		Note[A]	Note[A]	

Carbapenems[1]	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Doripenem	Note[1]	Note[1]		Note[A]	Note[A]	**1/A**. The susceptibility of streptococcus groups A, B, C and G to carbapenems is inferred from the benzylpenicillin susceptibility.
Ertapenem	Note[1]	Note[1]		Note[A]	Note[A]	
Imipenem	Note[1]	Note[1]		Note[A]	Note[A]	
Meropenem	Note[1]	Note[1]		Note[A]	Note[A]	

Monobactams	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Aztreonam	-	-		-	-	

Fluoroquinolones	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Ciprofloxacin	-	-		-	-	**A.** The norfloxacin disk diffusion test can be used to screen for fluoroquinolone resistance. **See Note B.**
Levofloxacin	2	2	5	17[A]	17[A]	**B.** Isolates categorised as susceptible to norfloxacin can be reported susceptible to levofloxacin and moxifloxacin. Isolates categorised as non-susceptible should be tested for susceptibility to individual agents.
Moxifloxacin	0.5	0.5	5	19[A]	19[A]	
Nalidixic acid (screen)	NA	NA		NA	NA	
Norfloxacin (screen)	NA	NA	10	12[B]	Note[B]	
Ofloxacin	-	-		-	-	

Aminoglycosides	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Amikacin	-	-		-	-	
Gentamicin	-	-		-	-	
Netilmicin	-	-		-	-	
Tobramycin	-	-		-	-	

Glycopeptides and lipoglycopeptides	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Dalbavancin[1]	0.125[2,3]	0.125[2]		Note[A]	Note[A]	**1.** Non-susceptible isolates are rare or not yet reported. The identification and antimicrobial susceptibility test result on any such isolate must be confirmed and the isolate sent to a reference laboratory.
Oritavancin[1]	0.25[2,3]	0.25[2]		Note[A]	Note[A]	**2.** MICs must be determined in the presence of polysorbate-80 (0.002% in the medium for broth dilution methods; agar dilution methods have not been validated). Follow the manufacturer's instructions for commercial systems.
Teicoplanin[1]	2	2	30	15	15	**3.** Isolates susceptible to vancomycin can be reported susceptible to dalbavancin and oritavancin.
Telavancin	IE	IE		IE	IE	
Vancomycin[1]	2	2	5	13	13	**A.** Disk diffusion criteria have not been defined and an MIC method should be used.

Macrolides, lincosamides and streptogramins	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Azithromycin	0.25[1]	0.5[1]		Note[A]	Note[A]	**1/A.** Erythromycin can be used to determine susceptibility to azithromycin, clarithromycin and roxithromycin.
Clarithromycin	0.25[1]	0.5[1]		Note[A]	Note[A]	**2.** Inducible clindamycin resistance can be detected by antagonism of clindamycin activity by a macrolide agent. If not detected, then report as susceptible. If detected, then report as resistant and consider adding this comment to the report: "Clindamycin may still be used for short-term therapy of less serious skin and soft tissue infections as constitutive resistance is unlikely to develop during such therapy". The clinical importance of inducible clindamycin resistance in combination treatment of severe *S. pyogenes* infections is not known.
Erythromycin	0.25[1]	0.5[1]	**15**	21[A]	18[A]	
Roxithromycin	0.5[1]	1[1]		Note[A]	Note[A]	
Telithromycin	0.25	0.5	**15**	20	17	
						B. Place the erythromycin and clindamycin disks 12-16 mm apart (edge to edge) and look for antagonism (the D phenomenon) to detect inducible clindamycin resistance.
Clindamycin[2]	0.5	0.5	**2**	17[B]	17[B]	
Quinupristin-dalfopristin	-	-		-	-	

Tetracyclines	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Doxycycline	1[1]	2[1]		Note[A]	Note[A]	**1/A.** Isolates susceptible to tetracycline are also susceptible to doxycycline and minocycline, but some resistant to tetracycline may be susceptible to minocycline and/or doxycycline. An MIC method should be used to test doxycycline susceptibility of tetracycline resistant isolates if required.
Minocycline	0.5[1]	1[1]	**30**	23[A]	20[A]	**2.** Non-susceptible isolates are rare or not yet reported. The identification and antimicrobial susceptibility test result on any such isolate must be confirmed and the isolate sent to a reference laboratory.
Tetracycline	1[1]	2[1]	**30**	23[A]	20[A]	**3.** For tigecycline broth microdilution MIC determination, the medium must be prepared fresh on the day of use.
Tigecycline[2]	0.25[3]	0.5[3]	**15**	19	16	

Oxazolidinones	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Linezolid[1]	2	4	**10**	19	16	**1.** Non-susceptible isolates are rare or not yet reported. The identification and antimicrobial susceptibility test result on any such isolate must be confirmed and the isolate sent to a reference laboratory.
Tedizolid[1]	0.5[2]	0.5		Note[A]	Note[A]	**2.** Isolates susceptible to linezolid can be reported susceptible to tedizolid.
						A. Isolates susceptible to linezolid can be reported susceptible to tedizolid. For isolates resistant to linezolid, perform an MIC test.

Miscellaneous agents	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Chloramphenicol	8	8	**30**	19	19	**1.** Non-susceptible isolates are rare or not yet reported. The identification and antimicrobial susceptibility test result on any such isolate must be confirmed and the isolate sent to a reference laboratory.
Colistin	-	-		-	-	
Daptomycin[1]	1[2]	1[2]		Note[A]	Note[A]	**2.** Daptomycin MICs must be determined in the presence of Ca^{2+} (50 mg/L in the medium for broth dilution methods; agar dilution methods have not been validated). Follow the manufacturer's instructions for commercial systems.
Fosfomycin iv	-	-		-	-	**3**. Trimethoprim-sulfamethoxazole in the ratio 1:19. Breakpoints are expressed as the trimethoprim concentration.
Fosfomycin oral	-	-		-	-	
Fusidic acid	IE	IE		IE	IE	**A.** Use an MIC method.
Metronidazole	-	-		-	-	
~~Mupirocin~~						
Nitrofurantoin (uncomplicated UTI only), *S. agalactiae* (group B streptococci)	64	64	**100**	15	15	
Nitroxoline (uncomplicated UTI only)	-	-		-	-	
Rifampicin	0.06	0.5	**5**	21	15	
Spectinomycin	-	-		-	-	
Trimethoprim (uncomplicated UTI only), *S. agalactiae* (group B streptococci)	2	2	**5**	IP	IP	
Trimethoprim-sulfamethoxazole[3]	1	2	**1.25-23.75**	18	15	

Streptococcus pneumoniae

Disk diffusion (EUCAST standardised disk diffusion method)
Medium: Mueller-Hinton agar + 5% defibrinated horse blood and 20 mg/L β-NAD (MH-F)
Inoculum: McFarland 0.5 from blood agar or McFarland 1.0 from chocolate agar
Incubation: 5% CO_2, 35±1ºC, 18±2h
Reading: Read zone edges as the point showing no growth viewed from the front of the plate with the lid removed and with reflected light.
Quality control: *Streptococcus pneumoniae* ATCC 49619

Penicillins[1]	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)	
	S ≤	R >		S ≥	R <
Benzylpenicillin (infections other than meningitis)[2]	0.06[1]	2[1]		Note[A]	Note[A]
Benzylpenicillin (meningitis)	0.06[1]	0.06[1]		Note[A]	Note[A]
Ampicillin	0.5[1,3]	2[1,3]		Note[A,B]	Note[A,B]
Ampicillin-sulbactam	Note[1,4]	Note[1,4]		Note[A,B]	Note[A,B]
Amoxicillin	Note[1,3,4]	Note[1,3,4]		Note[A,B]	Note[A,B]
Amoxicillin-clavulanic acid	Note[1,3,4]	Note[1,3,4]		Note[A,B]	Note[A,B]
Piperacillin	Note[1,4]	Note[1,4]		Note[A,B]	Note[A,B]
Piperacillin-tazobactam	Note[1,4]	Note[1,4]		Note[A,B]	Note[A,B]
Ticarcillin	-	-		-	-
Ticarcillin-clavulanic acid	-	-		-	-
Temocillin	-	-		-	-
Phenoxymethylpenicillin	Note[1]	Note[1]		Note[A]	Note[A]
Oxacillin (screen)	NA	NA	1	20[C]	Note[C]
Cloxacillin	-	-		-	-
Dicloxacillin	-	-		-	-
Flucloxacillin	-	-		-	-
Mecillinam (uncomplicated UTI only)	-	-		-	-

Notes
Numbered notes relate to general comments and/or MIC breakpoints.
Lettered notes relate to the disk diffusion method.

1. Breakpoints for penicillins other than benzylpenicillin relate only to non-meningitis isolates.
Isolates fully susceptible to benzylpenicillin (MIC ≤0.06 mg/L and/or susceptible by **oxacillin disk screen, see note C**) can be reported susceptible to beta-lactam agents for which clinical breakpoints are listed (including those with "Note").
2. In pneumonia, when a dose of 1.2 g x 4 is used, isolates with **MIC ≤0.5 mg/L** should be regarded as susceptible.
In pneumonia, when a dose of 2.4 g x 4 or 1.2 g x 6 is used, isolates with **MIC ≤1 mg/L** should be regarded as susceptible.
In pneumonia, when a dose of 2.4 g x 6 is used, isolates with **MIC ≤2 mg/L** should be regarded as susceptible.
3. For isolates categorised as intermediate to ampicillin avoid oral treatment with ampicillin, amoxicillin or amoxicillin-clavulanic acid.
4/B. Susceptibility inferred from the MIC of ampicillin.

A. Screen for beta-lactam resistance with the oxacillin 1 µg disk, **see Note C**.
C. For interpretation of the oxacillin disk screen, see flow chart below.
For oxacillin non-susceptible isolates, always determine the MIC of benzylpenicillin.

Cephalosporins	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Cefaclor	0.03	0.5	30	50	28	**A.** Screen for beta-lactam resistance with the oxacillin 1 µg disk. **See Note C on penicillins and flow chart below.**
Cefadroxil	-	-		-	-	
Cefalexin	-	-		-	-	
Cefazolin	-	-		-	-	
Cefepime	1	2		Note[A]	Note[A]	
Cefixime	-	-		-	-	
Cefotaxime	0.5	2		Note[A]	Note[A]	
Cefoxitin	NA	NA		NA	NA	
Cefpodoxime	0.25	0.5		Note[A]	Note[A]	
Ceftaroline	0.25	0.25		Note[A]	Note[A]	
Ceftazidime	-	-		-	-	
Ceftazidime-avibactam	-	-		-	-	
Ceftibuten	-	-		-	-	
Ceftobiprole	0.5	0.5		Note[A]	Note[A]	
Ceftolozane-tazobactam	-	-		-	-	
Ceftriaxone	0.5	2		Note[A]	Note[A]	
Cefuroxime iv	0.5	1		Note[A]	Note[A]	
Cefuroxime oral	0.25	0.5		Note[A]	Note[A]	

Carbapenems	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Doripenem[1]	1	1		Note[A]	Note[A]	**1.** Not for meningitis (meropenem is the only carbapenem used for meningitis).
Ertapenem[1]	0.5	0.5		Note[A]	Note[A]	**2.** Meropenem is the only carbapenem used for meningitis.
Imipenem[1]	2	2		Note[A]	Note[A]	
Meropenem[1] (infections other than meningitis)	2	2		Note[A]	Note[A]	**A.** Screen for beta-lactam resistance with the oxacillin 1 µg disk. **See Note C on penicillins and flow chart below.**
Meropenem[2] (meningitis)	0.25	1		Note[A,B]	Note[A,B]	**B.** For use in meningitis determine the meropenem MIC.

Monobactams	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Aztreonam	-	-		-	-	

Fluoroquinolones	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Ciprofloxacin	-	-		-	-	~~**1.** Wild type *S. pneumoniae* are not considered susceptible to ciprofloxacin and are therefore categorised as intermediate.~~
Levofloxacin[1]	2	2	**5**	16[A]	16[A]	**1.** Breakpoints are based on high dose therapy (0.5 g x 2).
Moxifloxacin	0.5	0.5	**5**	22[A]	22[A]	~~**3.** Wild type *S. pneumoniae* are not considered susceptible to ofloxacin and are therefore categorised as intermediate.~~
Nalidixic acid (screen)	NA	NA		NA	NA	**A.** The norfloxacin disk diffusion test can be used to screen for fluoroquinolone resistance. **See Note B.**
Norfloxacin (screen)	NA	NA	**10**	11[B]	Note[B]	**B.** Isolates categorised as susceptible to norfloxacin can be reported susceptible to levofloxacin and moxifloxacin. ~~and intermediate to ciprofloxacin and ofloxacin.~~ Isolates categorised as non-susceptible should be tested for susceptibility to individual agents.
Ofloxacin	-	-		-	-	

Aminoglycosides	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Amikacin	-	-		-	-	
Gentamicin	-	-		-	-	
Netilmicin	-	-		-	-	
Tobramycin	-	-		-	-	

Glycopeptides and lipoglycopeptides	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Dalbavancin	IE	IE		IE	IE	**1.** Non-susceptible isolates are rare or not yet reported. The identification and antimicrobial susceptibility test result on any such isolate must be confirmed and the isolate sent to a reference laboratory.
Oritavancin	IE	IE		IE	IE	
Teicoplanin[1]	2	2	**30**	17	17	
Telavancin	IE	IE		IE	IE	
Vancomycin[1]	2	2	**5**	16	16	

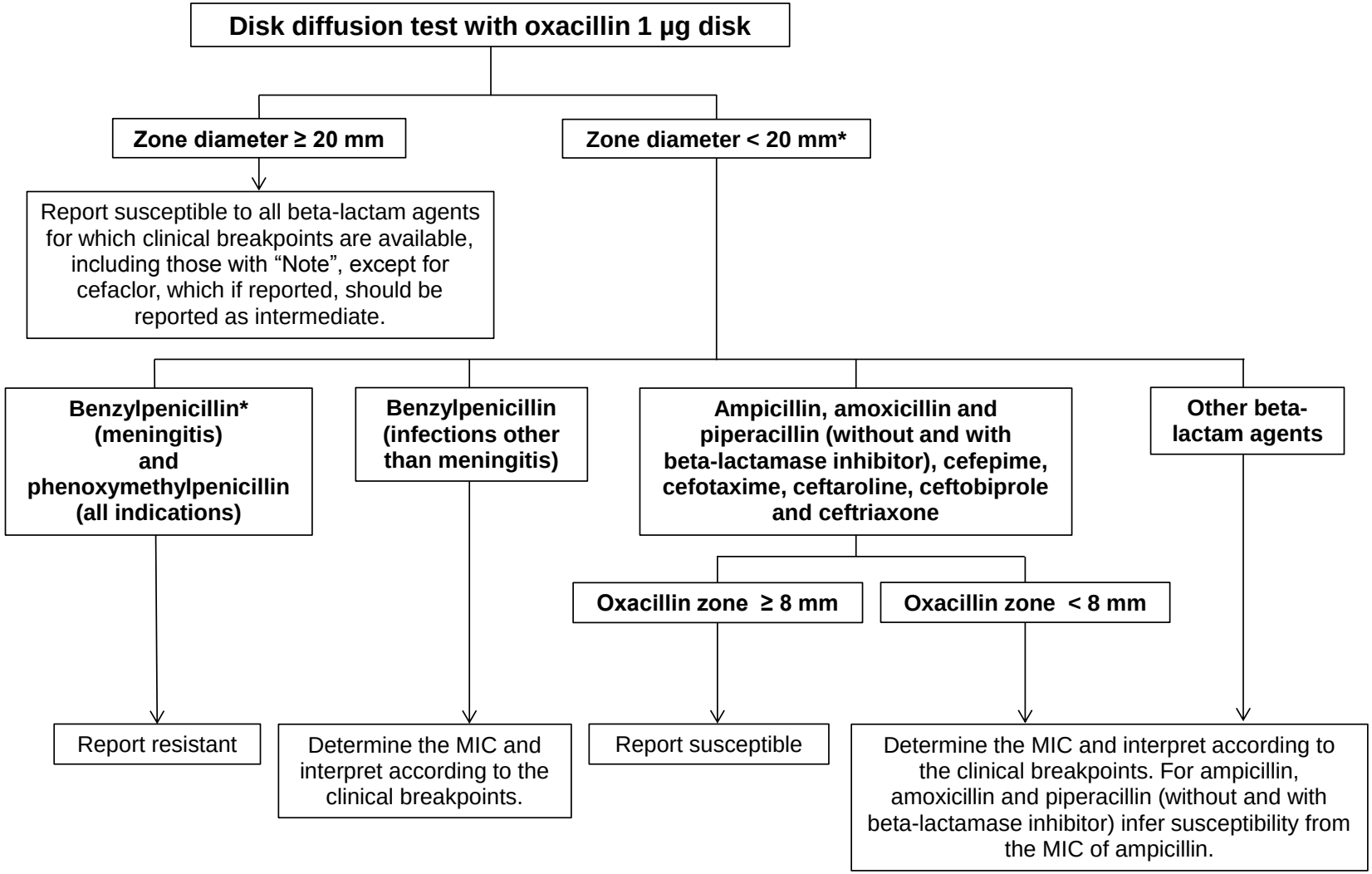
Macrolides, lincosamides and streptogramins	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
	S ≤	R >		S ≥	R <	
Azithromycin	0.25[1]	0.5[1]		Note[A]	Note[A]	**1/A.** Erythromycin can be used to determine susceptibility to azithromycin, clarithromycin and roxithromycin. **2.** Inducible clindamycin resistance can be detected by antagonism of clindamycin activity by a macrolide agent. If not detected, then report as susceptible. If detected, then report as resistant. **B.** Place the erythromycin and clindamycin disks 12-16 mm apart (edge to edge) and look for antagonism (the D phenomenon) to detect inducible clindamycin resistance.
Clarithromycin	0.25[1]	0.5[1]		Note[A]	Note[A]	
Erythromycin	0.25[1]	0.5[1]	**15**	22[A]	19[A]	
Roxithromycin	0.5[1]	1[1]		Note[A]	Note[A]	
Telithromycin	0.25	0.5	**15**	23	20	
Clindamycin[2]	0.5	0.5	**2**	19[B]	19[B]	
Quinupristin-dalfopristin	-	-		-	-	

Tetracyclines	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
	S ≤	R >		S ≥	R <	
Doxycycline	1[1]	2[1]		Note[A]	Note[A]	**1/A**. Isolates susceptible to tetracycline are also susceptible to doxycycline and minocycline, but some resistant to tetracycline may be susceptible to minocycline and/or doxycycline. An MIC method should be used to test doxycycline susceptibility of tetracycline resistant isolates if required.
Minocycline	0.5[1]	1[1]	**30**	24[A]	21[A]	
Tetracycline	1[1]	2[1]	**30**	25[A]	22[A]	
Tigecycline	IE	IE		IE	IE	

Oxazolidinones	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
	S ≤	R >		S ≥	R <	
Linezolid	2	4	**10**	22	19	
Tedizolid	IE	IE		IE	IE	

Miscellaneous agents	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
	S ≤	R >		S ≥	R <	
Chloramphenicol	8	8	**30**	21	21	**1.** Trimethoprim:sulfamethoxazole in the ratio 1:19. Breakpoints are expressed as the trimethoprim concentration.
Colistin	-	-		-	-	
Daptomycin	IE	IE		IE	IE	
Fosfomycin iv	IE	IE		IE	IE	
Fosfomycin oral	-	-		-	-	
Fusidic acid	-	-		-	-	
Metronidazole	-	-		-	-	
~~**Mupirocin**~~						
Nitrofurantoin (uncomplicated UTI only)	-	-		-	-	
Nitroxoline (uncomplicated UTI only)	-	-		-	-	
Rifampicin	0.06	0.5	**5**	22	17	
Spectinomycin	-	-		-	-	
Trimethoprim (uncomplicated UTI only)	-	-		-	-	
Trimethoprim-sulfamethoxazole[1]	1	2	**1.25-23.75**	18	15	

Screening for beta-lactam resistance in *S. pneumoniae*



*Always determine the MIC of benzylpenicillin. Do not delay reporting resistant in meningitis.

Viridans group streptococci

In endocarditis, refer to national or international endocarditis guidelines for breakpoints for viridans group streptococci.

Disk diffusion (EUCAST standardised disk diffusion method)
Medium: Mueller-Hinton agar + 5% defibrinated horse blood and 20 mg/L β-NAD (MH-F)
Inoculum: McFarland 0.5
Incubation: 5% CO_2, 35±1ºC, 18±2h
Reading: Read zone edges as the point showing no growth viewed from the front of the plate with the lid removed and with reflected light.
Quality control: *Streptococcus pneumoniae* ATCC 49619

This group of bacteria includes many species, which can be grouped as follows:
***S. anginosus* group:** *S. anginosus, S. constellatus, S. intermedius*
***S. mitis* group:** *S. australis, S. cristatus, S. infantis, S. mitis, S. oligofermentans, S. oralis,S. peroris, S. pseudopneumoniae, S. sinensis*
***S. sanguinis* group:** *S. sanguinis, S. parasanguinis, S. gordonii*
***S. bovis* group:** *S. equinus, S. gallolyticus (S. bovis), S. infantarius*
***S. salivarius* group:** *S. salivarius, S. vestibularis, S. thermophilus*
***S. mutans* group:** *S. mutans, S. sobrinus*

Penicillins	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Benzylpenicillin	0.25	2	1 unit	18	12	**1/B.** For isolates susceptible to benzylpenicillin, susceptibility can be inferred from benzylpenicillin or ampicillin. For isolates resistant to benzylpenicillin, susceptibility is inferred from ampicillin.
Benzylpenicillin (screen)	NA	NA	1 unit	18[A]	Note[A]	
Ampicillin	0.5	2	2	21	15	**A**. Benzylpenicillin 1 unit can be used to screen for beta-lactam resistance in viridans group streptococci. Isolates categorised as susceptible can be reported susceptible to beta-lactam agents for which clinical breakpoints are listed (including those with "Note"). Isolates categorised as non-susceptible should be tested for susceptibility to individual agents.
Ampicillin-sulbactam	Note[1]	Note[1]		Note[A,B]	Note[A,B]	
Amoxicillin	0.5	2		Note[A,B]	Note[A,B]	
Amoxicillin-clavulanic acid	Note[1]	Note[1]		Note[A,B]	Note[A,B]	
Piperacillin	Note[1]	Note[1]		Note[A,B]	Note[A,B]	
Piperacillin-tazobactam	Note[1]	Note[1]		Note[A,B]	Note[A,B]	
Ticarcillin	IE	IE		IE	IE	
Ticarcillin-clavulanic acid	IE	IE		IE	IE	
Temocillin	-	-		-	-	
Phenoxymethylpenicillin	IE	IE		IE	IE	
Oxacillin	-	-		-	-	
Cloxacillin	-	-		-	-	
Dicloxacillin	-	-		-	-	
Flucloxacillin	-	-		-	-	
Mecillinam (uncomplicated UTI only)	-	-		-	-	

Cephalosporins	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
	S ≤	R >		S ≥	R <	
Cefaclor	-	-		-	-	**A**. Benzylpenicillin 1 unit can be used to screen for beta-lactam resistance in viridans group streptococci. **See Note A on penicillins.**
Cefadroxil	-	-		-	-	
Cefalexin	-	-		-	-	
Cefazolin	0.5	0.5	**30**	IP	IP	
Cefepime	0.5	0.5	**30**	25[A]	25[A]	
Cefixime	-	-		-	-	
Cefotaxime	0.5	0.5	**5**	23[A]	23[A]	
Cefoxitin	NA	NA		NA	NA	
Cefpodoxime	-	-		-	-	
Ceftaroline	-	-		-	-	
Ceftazidime	-	-		-	-	
Ceftazidime-avibactam	-	-		-	-	
Ceftibuten	-	-		-	-	
Ceftobiprole	-	-		-	-	
Ceftolozane-tazobactam, *S. anginosus* group	IE	IE		IE	IE	
Ceftriaxone	0.5	0.5	**30**	27[A]	27[A]	
Cefuroxime iv	0.5	0.5	**30**	26[A]	26[A]	
Cefuroxime oral	-	-		-	-	

Carbapenems	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
	S ≤	R >		S ≥	R <	
Doripenem	1	1		Note[A]	Note[A]	**A**. Benzylpenicillin 1 unit can be used to screen for beta-lactam resistance in viridans group streptococci. **See Note A on penicillins.**
Ertapenem	0.5	0.5		Note[A]	Note[A]	
Imipenem	2	2		Note[A]	Note[A]	
Meropenem	2	2		Note[A]	Note[A]	

Monobactams	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
	S ≤	R >		S ≥	R <	
Aztreonam	-	-		-	-	

Fluoroquinolones	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Ciprofloxacin	-	-		-	-	
Levofloxacin	IE	IE		IE	IE	
Moxifloxacin	IE	IE		IE	IE	
Nalidixic acid (screen)	NA	NA		NA	NA	
Norfloxacin (uncomplicated UTI only)	-	-		-	-	
Ofloxacin	-	-		-	-	

Aminoglycosides[1]	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Amikacin	Note[2]	Note[2]		-	-	**1**. Viridans group streptococci are intrinsically resistant to aminoglycosides and aminoglycoside monotherapy is ineffective. There is likely to be synergy between aminoglycosides and penicillins or glycopeptides against streptococci without acquired high-level resistance. All testing is therefore to distinguish between intrinsic and high-level acquired resistance.
Gentamicin	Note[2]	Note[2]		-	-	**2.** Gentamicin can be used to screen for high-level aminoglycoside resistance (HLAR). **Negative test:** Isolates with gentamicin MIC ≤128 mg/L. The isolate is wild type for gentamicin and low-level intrinsic resistant. For other aminoglycosides, this may not be the case. Synergy with penicillins or glycopeptides can be expected if the isolate is susceptible to the penicillin or glycopeptide. **Positive test:** Isolates with gentamicin MIC >128 mg/L. The isolate is high-level resistant to gentamicin and other aminoglycosides except streptomycin. There will be no synergy with penicillins or glycopeptides.
Netilmicin	Note[2]	Note[2]		-	-	
Tobramycin	Note[2]	Note[2]		-	-	

Glycopeptides and lipoglycopeptides	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Dalbavancin, *S. anginosus* group[1]	0.125[2,3]	0.125[2]		Note[A]	Note[A]	**1.** Non-susceptible isolates are rare or not yet reported. The identification and antimicrobial susceptibility test result on any such isolate must be confirmed and the isolate sent to a reference laboratory.
Oritavancin, *S. anginosus* group[1]	0.25[2,3]	0.25[2]		Note[A]	Note[A]	**2.** MICs must be determined in the presence of polysorbate-80 (0.002% in the medium for broth dilution methods; agar dilution methods have not been validated). Follow the manufacturer's instructions for commercial systems.
Teicoplanin[1]	2	2	30	16	16	**3.** Isolates susceptible to vancomycin can be reported susceptible to dalbavancin and oritavancin.
Telavancin	IE	IE		IE	IE	
Vancomycin[1]	2	2	5	15	15	**A.** Disk diffusion criteria have not been defined and an MIC method should be used.

Macrolides, lincosamides and streptogramins	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
	S ≤	R >		S ≥	R <	
Azithromycin	IE	IE		IE	IE	**1.** Inducible clindamycin resistance can be detected by antagonism of clindamycin activity by a macrolide agent. If not detected, then report as susceptible. If detected, then report as resistant. **A.** Place the erythromycin and clindamycin disks 12-16 mm apart (edge to edge) and look for antagonism (the D phenomenon) to detect inducible clindamycin resistance.
Clarithromycin	IE	IE		IE	IE	
Erythromycin	IE	IE	**15**	IE	IE	
Roxithromycin	IE	IE		IE	IE	
Telithromycin	IE	IE		IE	IE	
Clindamycin[1]	0.5	0.5	**2**	19[A]	19[A]	
Quinupristin-dalfopristin	IE	IE		IE	IE	

Tetracyclines	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
	S ≤	R >		S ≥	R <	
Doxycycline	-	-		-	-	
Minocycline	-	-		-	-	
Tetracycline	-	-		-	-	
Tigecycline	IE	IE		IE	IE	

Oxazolidinones	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
	S ≤	R >		S ≥	R <	
Linezolid	-	-		-	-	**A.** Perform an MIC test.
Tedizolid, *S. anginosus* group	0.25	0.25		Note[A]	Note[A]	

Miscellaneous agents	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
	S ≤	R >		S ≥	R <	
Chloramphenicol	-	-		-	-	
Colistin	-	-		-	-	
Daptomycin	-	-		-	-	
Fosfomycin iv	-	-		-	-	
Fosfomycin oral	-	-		-	-	
Fusidic acid	-	-		-	-	
Metronidazole	-	-		-	-	
~~Mupirocin~~						
Nitrofurantoin (uncomplicated UTI only)	-	-		-	-	
Nitroxoline (uncomplicated UTI only)	-	-		-	-	
Rifampicin	-	-		-	-	
Spectinomycin	-	-		-	-	
Trimethoprim (uncomplicated UTI only)	-	-		-	-	
Trimethoprim-sulfamethoxazole	-	-		-	-	

EUCAST breakpoints have been defined for *H. influenzae* only. Clinical data for other *Haemophilus* species are scarce. MIC distributions for *H. parainfluenzae* are similar to those for *H. influenzae.* In the absence of specific breakpoints, the *H. influenzae* MIC breakpoints can be applied to *H. parainfluenzae.*

Disk diffusion (EUCAST standardised disk diffusion method)
Medium: Mueller-Hinton agar + 5% defibrinated horse blood and 20 mg/L β-NAD (MH-F)
Inoculum: McFarland 0.5
Incubation: 5% CO_2, 35±1ºC, 18±2h
Reading: Read zone edges as the point showing no growth viewed from the front of the plate with the lid removed and with reflected light.
Quality control: *Haemophilus influenzae* ATCC 49766. For control of the inhibitor component of beta-lactam inhibitor-combination disks, use *Staphylococcus aureus* ATCC 29213.

Penicillins	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)	
	S ≤	R >		S ≥	R <
Benzylpenicillin	IE	IE		IE	IE
Benzylpenicillin (screen)	NA	NA	1 unit	12[A]	Note[A]
Ampicillin[1,2]	1	1	2	16[A]	16[A]
Ampicillin-sulbactam[1]	1[3,4]	1[3,4]	10-10	Note[A,B]	Note[A,B]
Amoxicillin[1,2]	2	2		Note[A,C]	Note[A,C]
Amoxicillin-clavulanic acid[1]	2[5]	2[5]	2-1	15[A]	15[A]
Piperacillin[1,2]	Note[6]	Note[6]		Note[A,D]	Note[A,D]
Piperacillin-tazobactam[1]	Note[4]	Note[4]		Note[A,B]	Note[A,B]
Ticarcillin	IE	IE		IE	IE
Ticarcillin-clavulanic acid	IE	IE		IE	IE
Temocillin	IE	IE		IE	IE
Phenoxymethylpenicillin	IE	IE		IE	IE
Oxacillin	-	-		-	-
Cloxacillin	-	-		-	-
Dicloxacillin	-	-		-	-
Flucloxacillin	-	-		-	-
Mecillinam (uncomplicated UTI only)	-	-		-	-

Notes
Numbered notes relate to general comments and/or MIC breakpoints.
Lettered notes relate to the disk diffusion method.

1. Breakpoints are based on intravenous administration. ~~For penicillins without inhibitors, breakpoints apply to beta-lactamase negative isolates only. For penicillins without inhibitors, beta-lactamase positive isolates should be reported resistant.~~
2. Beta-lactamase positive isolates can be reported resistant to ampicillin, amoxicillin and piperacillin without inhibitors. Tests based on a chromogenic cephalosporin can be used to detect the beta-lactamase.
3. For susceptibility testing purposes, the concentration of sulbactam is fixed at 4 mg/L.
4/B. Susceptibility can be inferred from amoxicillin-clavulanic acid.
5. For susceptibility testing purposes, the concentration of clavulanic acid is fixed at 2 mg/L.
6/D. Susceptibility inferred from ampicillin or amoxicillin.

A. Benzylpenicillin 1 unit can be used to screen for, but not to distinguish between, beta-lactamase producing isolates and isolates with PBP mutations. **For interpretation of the benzylpenicillin disk screen, see flow chart below.**
C. Susceptibility can be inferred from ampicillin.

Cephalosporins	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Cefaclor	-	-		-	-	**A.** Benzylpenicillin 1 unit can be used to screen for beta-lactam resistance. **See Note A on penicillins and flow chart below.**
Cefadroxil	-	-		-	-	
Cefalexin	-	-		-	-	
Cefazolin	-	-		-	-	
Cefepime	0.25	0.25	30	28[A]	28[A]	
Cefixime	0.125	0.125	5	26[A]	26[A]	
Cefotaxime	0.125	0.125	5	27[A]	27[A]	
Cefoxitin	NA	NA		NA	NA	
Cefpodoxime	0.25	0.5	10	26[A]	23[A]	
Ceftaroline	0.03	0.03		Note[A]	Note[A]	
Ceftazidime	-	-		-	-	
Ceftazidime-avibactam	-	-		-	-	
Ceftibuten	1	1	30	25[A]	25[A]	
Ceftobiprole	IE	IE		IE	IE	
Ceftolozane-tazobactam	IE	IE		IE	IE	
Ceftriaxone	0.125	0.125	30	31[A]	31[A]	
Cefuroxime iv	1	2	30	26[A]	25[A]	
Cefuroxime oral	0.125	1	30	50	26	

Carbapenems	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Doripenem[1]	1	1	10	20[A]	20[A]	**1.** Not for meningitis (meropenem is the only carbapenem used for meningitis).
Ertapenem[1]	0.5	0.5	10	20[A]	20[A]	**2.** Meropenem is the only carbapenem used for meningitis.
Imipenem[1]	2	2	10	20[A]	20[A]	
Meropenem[1] (infections other than meningitis)	2	2	10	20[A]	20[A]	**A.** Benzylpenicillin 1 unit can be used to screen for beta-lactam resistance. **See Note A on penicillins and flow chart below.**
Meropenem[2] (meningitis)	0.25	1		Note[B]	Note[B]	**B.** For use in meningitis determine the meropenem MIC value.

Monobactams	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Aztreonam	IE	IE		IE	IE	

Fluoroquinolones	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Ciprofloxacin	0.06	0.06	5	30[A]	30[A]	~~1. Low-level fluoroquinolone resistance (ciprofloxacin MICs of 0.125-0.5 mg/L) may occur but there is no evidence that this resistance is of clinical importance in respiratory tract infections with *H. influenzae* .~~
Levofloxacin	0.06	0.06	5	30[A]	30[A]	
Moxifloxacin	0.125	0.125	5	28[A]	28[A]	**A.** The nalidixic acid disk diffusion test can be used to screen for fluoroquinolone resistance. **See Note B.**
Nalidixic acid (screen)	NA	NA	30	23[B]	Note[B]	**B.** Isolates categorised as susceptible to nalidixic acid can be reported susceptible to ciprofloxacin, levofloxacin, moxifloxacin and ofloxacin. Isolates categorised as non-susceptible may have fluoroquinolone resistance and should be tested against the appropriate agent.
Norfloxacin (uncomplicated UTI only)	-	-		-	-	
Ofloxacin	0.06	0.06	5	30[A]	30[A]	

Aminoglycosides	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Amikacin	IE	IE		IE	IE	
Gentamicin	IE	IE		IE	IE	
Netilmicin	IE	IE		IE	IE	
Tobramycin	IE	IE		IE	IE	

Glycopeptides and lipoglycopeptides	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Dalbavancin	-	-		-	-	
Oritavancin	-	-		-	-	
Teicoplanin	-	-		-	-	
Telavancin	-	-		-	-	
Vancomycin	-	-		-	-	

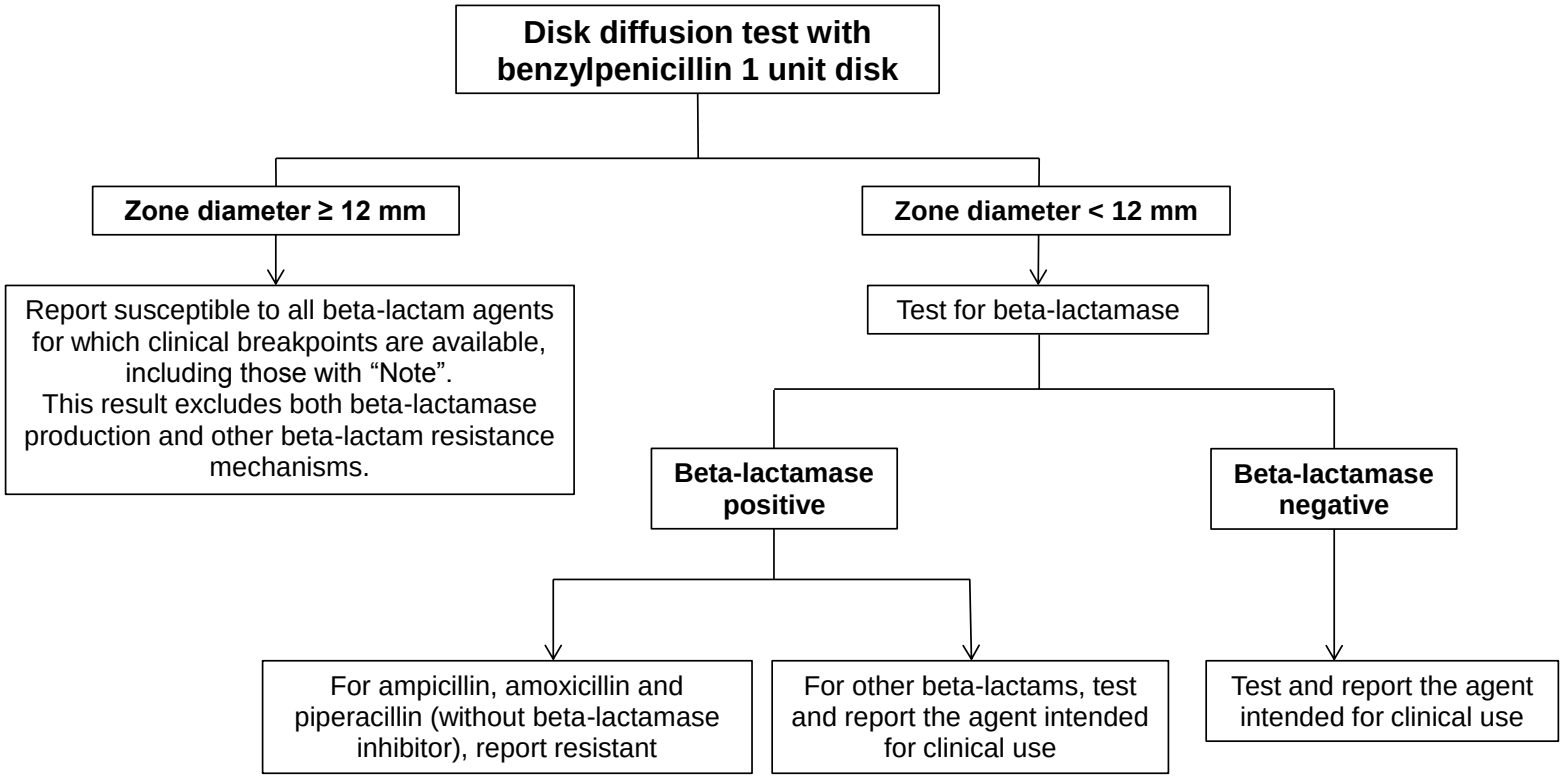
Macrolides[1], lincosamides and streptogramins	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Azithromycin	0.125[2]	4[2]		Note[A]	Note[A]	**1.** Correlation between macrolide MICs and clinical outcome is weak for *H. influenzae* . Therefore, breakpoints for macrolides and related antibiotics have been set to categorise wild type *H. influenzae* as intermediate.
Clarithromycin	1[2]	32[2]		Note[A]	Note[A]	**2/A.** Erythromycin can be used to determine susceptibility to azithromycin, clarithromycin and roxithromycin.
Erythromycin	0.5	16	15	50	10	
Roxithromycin	1[2]	16[2]		Note[A]	Note[A]	
Telithromycin	0.125	8	15	50	12	
	-	-		-	-	
Clindamycin	-	-		-	-	
Quinupristin-dalfopristin	-	-		-	-	

Tetracyclines	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
	S ≤	R >		S ≥	R <	
Doxycycline	1[1]	2[1]		Note[A]	Note[A]	**1/A**. Isolates susceptible to tetracycline are also susceptible to doxycycline and minocycline, but some resistant to tetracycline may be susceptible to minocycline and/or doxycycline. An MIC method should be used to test doxycycline susceptibility of tetracycline resistant isolates if required.
Minocycline	1[1]	2[1]	30	24[A]	21[A]	
Tetracycline	1[1]	2[1]	30	25[A]	22[A]	
Tigecycline	IE	IE		IE	IE	

Oxazolidinones	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
	S ≤	R >		S ≥	R <	
Linezolid	-	-		-	-	
Tedizolid	-	-		-	-	

Miscellaneous agents	MIC breakpoint (mg/L)			Zone diameter breakpoint (mm)		Notes Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
	S ≤	R >		S ≥	R <	
Chloramphenicol	2	2	30	28	28	**1.** Trimethoprim:sulfamethoxazole in the ratio 1:19. Breakpoints are expressed as the trimethoprim concentration.
Colistin	-	-		-	-	
Daptomycin	-	-		-	-	
Fosfomycin iv	IE	IE		IE	IE	
Fosfomycin oral	-	-		-	-	
Fusidic acid	-	-		-	-	
Metronidazole	-	-		-	-	
~~Mupirocin~~						
Nitrofurantoin (uncomplicated UTI only)	-	-		-	-	
Nitroxoline (uncomplicated UTI only)	-	-		-	-	
Rifampicin (for prophylaxis only)	1	1	5	18	18	
Spectinomycin	-	-		-	-	
Trimethoprim (uncomplicated UTI only)	-	-		-	-	
Trimethoprim-sulfamethoxazole[1]	0.5	1	1.25-23.75	23	20	

Screening for beta-lactam resistance in *H. influenzae*



Disk diffusion (EUCAST standardised disk diffusion method)
Medium: Mueller-Hinton agar + 5% defibrinated horse blood and 20 mg/L β-NAD (MH-F)
Inoculum: McFarland 0.5
Incubation: 5% CO_2, 35±1ºC, 18±2h
Reading: Read zone edges as the point showing no growth viewed from the front of the plate with the lid removed and with reflected light.
Quality control: *Haemophilus influenzae* ATCC 49766. For control of the inhibitor component of beta-lactam inhibitor-combination disks, use *Staphylococcus aureus* ATCC 29213.

Penicillins	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Benzylpenicillin	-	-		-	-	**1.** Most *M. catarrhalis* produce beta-lactamase, although beta-lactamase production is slow and may give weak results with *in vitro* tests. Beta-lactamase producers should be reported resistant to penicillins and aminopenicillins without inhibitors.
Ampicillin	-[1]	-[1]		-	-	**2.** For susceptibility testing purposes, the concentration of sulbactam is fixed at 4 mg/L.
Ampicillin-sulbactam	1[2,3]	1[2,3]		Note[A]	Note[A]	**3/A.** Susceptibility can be inferred from amoxicillin-clavulanic acid.
Amoxicillin	-[1]	-[1]		-	-	**4.** For susceptibility testing purposes, the concentration of clavulanic acid is fixed at 2 mg/L.
Amoxicillin-clavulanic acid	1[4]	1[4]	2-1	19	19	
Piperacillin	-[1]	-[1]		-	-	
Piperacillin-tazobactam	Note[3]	Note[3]		Note[A]	Note[A]	
Ticarcillin	IE	IE		IE	IE	
Ticarcillin-clavulanic acid	IE	IE		IE	IE	
Temocillin	IE	IE		IE	IE	
Phenoxymethylpenicillin	-	-		-	-	
Oxacillin	-	-		-	-	
Cloxacillin	-	-		-	-	
Dicloxacillin	-	-		-	-	
Flucloxacillin	-	-		-	-	
Mecillinam (uncomplicated UTI only)	-	-		-	-	

Cephalosporins	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Cefaclor	-	-		-	-	
Cefadroxil	-	-		-	-	
Cefalexin	-	-		-	-	
Cefazolin	-	-		-	-	
Cefepime	4	4	30	20	20	
Cefixime	0.5	1	5	21	18	
Cefotaxime	1	2	5	20	17	
Cefoxitin	NA	NA		NA	NA	
Cefpodoxime	IP	IP	10	IP	IP	
Ceftaroline	IE	IE		IE	IE	
Ceftazidime	-	-		-	-	
Ceftazidime-avibactam	-	-		-	-	
Ceftibuten	IE	IE		IE	IE	
Ceftobiprole	IE	IE		IE	IE	
Ceftolozane-tazobactam	IE	IE		IE	IE	
Ceftriaxone	1	2	30	24	21	
Cefuroxime iv	4	8	30	21	18	
Cefuroxime oral	0.125	4	30	50	21	

Carbapenems	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Doripenem[1]	1	1	10	30	30	**1.** Non-susceptible isolates are rare or not yet reported. The identification and antimicrobial susceptibility test result on any such isolate must be confirmed and the isolate sent to a reference laboratory.
Ertapenem[1]	0.5	0.5	10	29	29	
Imipenem[1]	2	2	10	29	29	
Meropenem[1]	2	2	10	33	33	

Monobactams	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Aztreonam	IE	IE		IE	IE	

Fluoroquinolones	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Ciprofloxacin	0.5	0.5	5	26[A]	26[A]	**A.** The nalidixic acid disk diffusion test can be used to screen for fluoroquinolone resistance. **See Note B.**
Levofloxacin	1	1	5	26[A]	26[A]	**B.** Isolates categorised as susceptible to nalidixic acid can be reported susceptible to ciprofloxacin, levofloxacin, moxifloxacin and ofloxacin. Isolates categorised as non-susceptible may have fluoroquinolone resistance and should be tested against the appropriate agent.
Moxifloxacin	0.5	0.5	5	23[A]	23[A]	
Nalidixic acid (screen)	NA	NA	30	23[B]	Note[B]	
Norfloxacin (uncomplicated UTI only)	-	-		-	-	
Ofloxacin	0.5	0.5	5	25[A]	25[A]	

Aminoglycosides	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Amikacin	IE	IE		IE	IE	
Gentamicin	IE	IE		IE	IE	
Netilmicin	IE	IE		IE	IE	
Tobramycin	IE	IE		IE	IE	

Glycopeptides and lipoglycopeptides	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Dalbavancin	-	-		-	-	
Oritavancin	-	-		-	-	
Teicoplanin	-	-		-	-	
Telavancin	-	-		-	-	
Vancomycin	-	-		-	-	

Macrolides, lincosamides and streptogramins	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Azithromycin	0.25[1]	0.5[1]		Note[A]	Note[A]	**1/A.** Erythromycin can be used to determine susceptibility to azithromycin, clarithromycin and roxithromycin.
Clarithromycin	0.25[1]	0.5[1]		Note[A]	Note[A]	
Erythromycin	0.25	0.5	15	23[A]	20[A]	
Roxithromycin	0.5[1]	1[1]		Note[A]	Note[A]	
Telithromycin	0.25	0.5	15	23	20	
Clindamycin	-	-		-	-	
Quinupristin-dalfopristin	-	-		-	-	

Tetracyclines	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Doxycycline	1[1]	2[1]		Note[A]	Note[A]	**1/A**. Isolates susceptible to tetracycline are also susceptible to doxycycline and minocycline, but some resistant to tetracycline may be susceptible to minocycline and/or doxycycline. An MIC method should be used to test doxycycline susceptibility of tetracycline resistant isolates if required.
Minocycline	1[1]	2[1]	30	25[A]	22[A]	
Tetracycline	1[1]	2[1]	30	28[A]	25[A]	
Tigecycline	IE	IE		IE	IE	

Oxazolidinones	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Linezolid	-	-		-	-	
Tedizolid	-	-		-	-	

Miscellaneous agents	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Chloramphenicol	2[1]	2[1]	30	30[A]	30[A]	**1/A**. Breakpoints relate to topical use only.
Colistin	-	-		-	-	**2.** Trimethoprim:sulfamethoxazole in the ratio 1:19. Breakpoints are expressed as the trimethoprim concentration.
Daptomycin	-	-		-	-	
Fosfomycin iv	IE	IE		IE	IE	
Fosfomycin oral	-	-		-	-	
Fusidic acid	-	-		-	-	
Metronidazole	-	-		-	-	
~~Mupirocin~~						
Nitrofurantoin (uncomplicated UTI only)	-	-		-	-	
Nitroxoline (uncomplicated UTI only)	-	-		-	-	
Rifampicin	-	-		-	-	
Spectinomycin	-	-		-	-	
Trimethoprim (uncomplicated UTI only)	-	-		-	-	
Trimethoprim-sulfamethoxazole[2]	0.5	1	1.25-23.75	18	15	

Disk diffusion criteria for antimicrobial susceptibility testing of *Neisseria gonorrhoeae* have not yet been defined and an MIC method should be used. If a commercial MIC method is used, follow the manufacturer´s instructions. Laboratories with few isolates are encouraged to refer these to a reference laboratory for testing.

Penicillins[1]	MIC breakpoint (mg/L)		Notes Numbered notes relate to general comments and/or MIC breakpoints.
	S ≤	R >	
Benzylpenicillin	0.06[1]	1	**1.** Always test for beta-lactamase. If positive, report resistant to benzylpenicillin, ampicillin and amoxicillin. Tests based on a chromogenic cephalosporin can be used to detect the beta-lactamase. The susceptibility of beta-lactamase negative isolates to ampicillin and amoxicillin can be inferred from benzylpenicillin.
Ampicillin[1]	Note[1]	Note[1]	
Ampicillin-sulbactam	IE	IE	
Amoxicillin[1]	Note[1]	Note[1]	
Amoxicillin-clavulanic acid	Note[1]	Note[1]	
Piperacillin	-	-	
Piperacillin-tazobactam	-	-	
Ticarcillin	-	-	
Ticarcillin-clavulanic acid	-	-	
Temocillin	IE	IE	
Phenoxymethylpenicillin	-	-	
Oxacillin	-	-	
Cloxacillin	-	-	
Dicloxacillin	-	-	
Flucloxacillin	-	-	
Mecillinam (uncomplicated UTI only)	-	-	

Cephalosporins	MIC breakpoint (mg/L)		Notes Numbered notes relate to general comments and/or MIC breakpoints.
	S ≤	R >	
Cefaclor	-	-	
Cefadroxil	-	-	
Cefalexin	-	-	
Cefazolin	-	-	
Cefepime	-	-	
Cefixime	0.125	0.125	
Cefotaxime	0.125	0.125	
Cefoxitin	-	-	
Cefpodoxime	-	-	
Ceftaroline	-	-	
Ceftazidime	-	-	
Ceftazidime-avibactam	-	-	
Ceftibuten	-	-	
Ceftobiprole	-	-	
Ceftolozane-tazobactam	-	-	
Ceftriaxone	0.125	0.125	
Cefuroxime iv	-	-	
Cefuroxime oral	-	-	

Carbapenems	MIC breakpoint (mg/L)		Notes Numbered notes relate to general comments and/or MIC breakpoints.
	S ≤	R >	
Doripenem	IE	IE	
Ertapenem	IE	IE	
Imipenem	IE	IE	
Meropenem	IE	IE	

Monobactams	MIC breakpoint (mg/L)		Notes Numbered notes relate to general comments and/or MIC breakpoints.
	S ≤	R >	
Aztreonam	IE	IE	

Fluoroquinolones	MIC breakpoint (mg/L)		Notes
	S ≤	R >	Numbered notes relate to general comments and/or MIC breakpoints.
Ciprofloxacin	0.03	0.06	
Levofloxacin	IE	IE	
Moxifloxacin	IE	IE	
Nalidixic acid (screen)	NA	NA	
Norfloxacin (uncomplicated UTI only)	-	-	
Ofloxacin	0.125	0.25	

Aminoglycosides	MIC breakpoint (mg/L)		Notes
	S ≤	R >	Numbered notes relate to general comments and/or MIC breakpoints.
Amikacin	-	-	
Gentamicin	-	-	
Netilmicin	-	-	
Tobramycin	-	-	

Glycopeptides and lipoglycopeptides	MIC breakpoint (mg/L)		Notes
	S ≤	R >	Numbered notes relate to general comments and/or MIC breakpoints.
Dalbavancin	-	-	
Oritavancin	-	-	
Teicoplanin	-	-	
Telavancin	-	-	
Vancomycin	-	-	

Macrolides, lincosamides and streptogramins	MIC breakpoint (mg/L)		Notes
	S ≤	R >	Numbered notes relate to general comments and/or MIC breakpoints.
Azithromycin[1]	0.25	0.5	**1.** Breakpoints are based on a 2 g-single dose in monotherapy.
Clarithromycin	-	-	
Erythromycin	-	-	
Roxithromycin	-	-	
Telithromycin	-	-	
Clindamycin	-	-	
Quinupristin-dalfopristin	-	-	

Tetracyclines	MIC breakpoint (mg/L)		Notes Numbered notes relate to general comments and/or MIC breakpoints.
	S ≤	R >	
Doxycycline	IE	IE	~~1. Isolates susceptible to tetracycline are also susceptible to minocycline, but some isolates resistant to tetracycline may be susceptible to minocycline.~~
Minocycline	IE	IE	
Tetracycline	0.5	1	
Tigecycline	IE	IE	

Oxazolidinones	MIC breakpoint (mg/L)		Notes Numbered notes relate to general comments and/or MIC breakpoints.
	S ≤	R >	
Linezolid	-	-	
Tedizolid	-	-	

Miscellaneous agents	MIC breakpoint (mg/L)		Notes Numbered notes relate to general comments and/or MIC breakpoints.
	S ≤	R >	
Chloramphenicol	-	-	
Colistin	-	-	
Daptomycin	-	-	
Fosfomycin iv	-	-	
Fosfomycin oral	-	-	
Fusidic acid	-	-	
Metronidazole	-	-	
~~Mupirocin~~			
Nitrofurantoin (uncomplicated UTI only)	-	-	
Nitroxoline (uncomplicated UTI only)	-	-	
Rifampicin	-	-	
Spectinomycin	64	64	
Trimethoprim (uncomplicated UTI only)	-	-	
Trimethoprim-sulfamethoxazole	-	-	

Disk diffusion criteria for antimicrobial susceptibility testing of *Neisseria meningitidis* have not yet been defined and an MIC method should be used. If a commercial MIC method is used, follow the manufacturer´s instructions.

Penicillins	MIC breakpoint (mg/L)		Notes Numbered notes relate to general comments and/or MIC breakpoints.
	S ≤	R >	
Benzylpenicillin	0.06	0.25	
Ampicillin	0.125	1	
Ampicillin-sulbactam	IE	IE	
Amoxicillin	0.125	1	
Amoxicillin-clavulanic acid	-	-	
Piperacillin	-	-	
Piperacillin-tazobactam	-	-	
Ticarcillin	-	-	
Ticarcillin-clavulanic acid	-	-	
Temocillin	-	-	
Phenoxymethylpenicillin	-	-	
Oxacillin	-	-	
Cloxacillin	-	-	
Dicloxacillin	-	-	
Flucloxacillin	-	-	
Mecillinam (uncomplicated UTI only)	-	-	

Cephalosporins	MIC breakpoint (mg/L)		Notes Numbered notes relate to general comments and/or MIC breakpoints.
	S ≤	R >	
Cefaclor	-	-	**1.** Non-susceptible isolates are rare or not yet reported. The identification and antimicrobial susceptibility test result on any such isolate must be confirmed and the isolate sent to a reference laboratory.
Cefadroxil	-	-	
Cefalexin	-	-	
Cefazolin	-	-	
Cefepime	-	-	
Cefixime	-	-	
Cefotaxime[1]	0.125	0.125	
Cefoxitin	-	-	
Cefpodoxime	-	-	
Ceftaroline	-	-	
Ceftazidime	-	-	
Ceftazidime-avibactam	-	-	
Ceftibuten	-	-	
Ceftobiprole	-	-	
Ceftolozane-tazobactam	-	-	
Ceftriaxone[1]	0.125	0.125	
Cefuroxime iv	-	-	
Cefuroxime oral	-	-	

Carbapenems	MIC breakpoint (mg/L)		Notes Numbered notes relate to general comments and/or MIC breakpoints.
	S ≤	R >	
Doripenem	IE	IE	~~**1.** Breakpoints relate to meningitis only.~~
Ertapenem	-	-	**1.** Non-susceptible isolates are rare or not yet reported. The identification and antimicrobial susceptibility test result on any such isolate must be confirmed and the isolate sent to a reference laboratory.
Imipenem	-	-	
Meropenem[1] (meningitis)	0.25	0.25	

Monobactams	MIC breakpoint (mg/L)		Notes Numbered notes relate to general comments and/or MIC breakpoints.
	S ≤	R >	
Aztreonam	-	-	

Fluoroquinolones	MIC breakpoint (mg/L)		Notes Numbered notes relate to general comments and/or MIC breakpoints.
	S ≤	R >	
Ciprofloxacin	0.03[1]	0.03[1]	**1.** Breakpoints apply only to use in the prophylaxis of meningococcal disease.
Levofloxacin	IE	IE	
Moxifloxacin	IE	IE	
Nalidixic acid (screen)	NA	NA	
Norfloxacin (uncomplicated UTI only)	-	-	
Ofloxacin	IE	IE	

Aminoglycosides	MIC breakpoint (mg/L)		Notes Numbered notes relate to general comments and/or MIC breakpoints.
	S ≤	R >	
Amikacin	-	-	
Gentamicin	-	-	
Netilmicin	-	-	
Tobramycin	-	-	

Glycopeptides and lipoglycopeptides	MIC breakpoint (mg/L)		Notes Numbered notes relate to general comments and/or MIC breakpoints.
	S ≤	R >	
Dalbavancin	-	-	
Oritavancin	-	-	
Teicoplanin	-	-	
Telavancin	-	-	
Vancomycin	-	-	

Macrolides, lincosamides and streptogramins	MIC breakpoint (mg/L)		Notes Numbered notes relate to general comments and/or MIC breakpoints.
	S ≤	R >	
Azithromycin	-	-	
Clarithromycin	-	-	
Erythromycin	-	-	
Roxithromycin	-	-	
Telithromycin	-	-	
Clindamycin	-	-	
Quinupristin-dalfopristin	-	-	

Tetracyclines	MIC breakpoint (mg/L)		Notes Numbered notes relate to general comments and/or MIC breakpoints.
	S ≤	R >	
Doxycycline	-	-	**1.** Tetracycline can be used to predict susceptibility to minocycline for prophylaxis against *N. meningitidis* infections.
Minocycline	1[1]	2[1]	
Tetracycline	1[1]	2[1]	
Tigecycline	IE	IE	

Oxazolidinones	MIC breakpoint (mg/L)		Notes Numbered notes relate to general comments and/or MIC breakpoints.
	S ≤	R >	
Linezolid	-	-	
Tedizolid	-	-	

Miscellaneous agents	MIC breakpoint (mg/L)		Notes Numbered notes relate to general comments and/or MIC breakpoints.
	S ≤	R >	
Chloramphenicol	2	4	**1.** For prophylaxis of meningitis only (refer to national guidelines).
Colistin	-	-	
Daptomycin	-	-	
Fosfomycin iv	-	-	
Fosfomycin oral	-	-	
Fusidic acid	-	-	
Metronidazole	-	-	
~~Mupirocin~~			
Nitrofurantoin (uncomplicated UTI only)	-	-	
Nitroxoline (uncomplicated UTI only)	-	-	
Rifampicin[1]	0.25	0.25	
Spectinomycin	-	-	
Trimethoprim (uncomplicated UTI only)	-	-	
Trimethoprim-sulfamethoxazole	-	-	

Gram-positive anaerobes

except *Clostridium difficile*

Disk diffusion criteria for antimicrobial susceptibility testing of anaerobes have not yet been defined and an MIC method should be used. If a commercial MIC method is used, follow the manufacturer´s instructions.

This group of bacteria includes many genera. The most frequently isolated Gram-positive anaerobes are: *Clostridium, Actinomyces, Propionibacterium, Bifidobacterium, Eggerthella, Eubacterium, Lactobacillu* s and anaerobic gram-positive cocci.
Anaerobes are most frequently defined by no growth on culture plates incubated in a CO_2 enriched atmosphere, but many Gram-positive, non-spore forming rods such as *Actinomyces* spp., many *P. acnes* and some *Bifidobacterium* spp. can grow on incubation in CO_2 and may be tolerant enough to grow poorly in air, but are still considered as anaerobic bacteria. Several species of *Clostridium,* including *C. carnis, C. histolyticum* and *C. tertium,* can grow but not sporulate in air.
For all these species, susceptibility testing should be performed in anaerobic environment.

Penicillins	MIC breakpoint (mg/L)		Notes Numbered notes relate to general comments and/or MIC breakpoints.
	S ≤	R >	
Benzylpenicillin[1]	0.25	0.5	**1.** Susceptibility to ampicillin, amoxicillin, piperacillin and ticarcillin can be inferred from susceptibility to benzylpenicillin.
Ampicillin[1]	4	8	**2.** For susceptibility testing purposes, the concentration of sulbactam is fixed at 4 mg/L.
Ampicillin-sulbactam	4[2]	8[2]	**3.** For susceptibility testing purposes, the concentration of clavulanic acid is fixed at 2 mg/L.
Amoxicillin[1]	4	8	**4.** For susceptibility testing purposes, the concentration of tazobactam is fixed at 4 mg/L.
Amoxicillin-clavulanic acid	4[3]	8[3]	
Piperacillin[1]	8	16	
Piperacillin-tazobactam	8[4]	16[4]	
Ticarcillin[1]	8	16	
Ticarcillin-clavulanic acid	8[3]	16[3]	
Temocillin	-	-	
Phenoxymethylpenicillin	IE	IE	
Oxacillin	-	-	
Cloxacillin	-	-	
Dicloxacillin	-	-	
Flucloxacillin	-	-	
Mecillinam (uncomplicated UTI only)	-	-	

Gram-positive anaerobes

except *Clostridium difficile*

Cephalosporins	MIC breakpoint (mg/L)		Notes Numbered notes relate to general comments and/or MIC breakpoints.
	S ≤	R >	
Cefaclor	-	-	
Cefadroxil	-	-	
Cefalexin	-	-	
Cefazolin	-	-	
Cefepime	-	-	
Cefixime	-	-	
Cefotaxime	-	-	
Cefoxitin	IE	IE	
Cefpodoxime	-	-	
Ceftaroline	-	-	
Ceftazidime	-	-	
Ceftazidime-avibactam	-	-	
Ceftibuten	-	-	
Ceftobiprole	-	-	
Ceftolozane-tazobactam	IE	IE	
Ceftriaxone	-	-	
Cefuroxime iv	-	-	
Cefuroxime oral	-	-	

Carbapenems	MIC breakpoint (mg/L)		Notes Numbered notes relate to general comments and/or MIC breakpoints.
	S ≤	R >	
Doripenem	1	1	
Ertapenem	1	1	
Imipenem	2	8	
Meropenem	2	8	

Monobactams	MIC breakpoint (mg/L)		Notes Numbered notes relate to general comments and/or MIC breakpoints.
	S ≤	R >	
Aztreonam	-	-	

Gram-positive anaerobes

except *Clostridium difficile*

Fluoroquinolones	MIC breakpoint (mg/L)		Notes
	S ≤	R >	Numbered notes relate to general comments and/or MIC breakpoints.
Ciprofloxacin	-	-	
Levofloxacin	-	-	
Moxifloxacin	IE	IE	
Nalidixic acid (screen)	NA	NA	
Norfloxacin (uncomplicated UTI only)	-	-	
Ofloxacin	-	-	

Aminoglycosides	MIC breakpoint (mg/L)		Notes
	S ≤	R >	Numbered notes relate to general comments and/or MIC breakpoints.
Amikacin	-	-	
Gentamicin	-	-	
Netilmicin	-	-	
Tobramycin	-	-	

Glycopeptides and lipoglycopeptides	MIC breakpoint (mg/L)		Notes
	S ≤	R >	Numbered notes relate to general comments and/or MIC breakpoints.
Dalbavancin	IE	IE	
Oritavancin	IE	IE	
Teicoplanin	IE	IE	
Telavancin	IE	IE	
Vancomycin	2	2	

Gram-positive anaerobes

except *Clostridium difficile*

Macrolides, lincosamides and streptogramins	MIC breakpoint (mg/L)		Notes Numbered notes relate to general comments and/or MIC breakpoints.
	S ≤	R >	
Azithromycin	-	-	
Clarithromycin	-	-	
Erythromycin	IE	IE	
Roxithromycin	-	-	
Telithromycin	-	-	
Clindamycin	4	4	
Quinupristin-dalfopristin	-	-	

Tetracyclines[1]	MIC breakpoint (mg/L)		Notes Numbered notes relate to general comments and/or MIC breakpoints.
	S ≤	R >	
Doxycycline	Note[1]	Note[1]	**1.** For anaerobic bacteria there is clinical evidence of activity in mixed intra-abdominal infections, but no correlation between MIC values, PK/PD data and clinical outcome. Therefore no breakpoints for susceptibility testing are given.
Minocycline	Note[1]	Note[1]	
Tetracycline	Note[1]	Note[1]	
Tigecycline	Note[1]	Note[1]	

Oxazolidinones	MIC breakpoint (mg/L)		Notes Numbered notes relate to general comments and/or MIC breakpoints.
	S ≤	R >	
Linezolid	-	-	
Tedizolid	-	-	

Gram-positive anaerobes

except *Clostridium difficile*

Miscellaneous agents	MIC breakpoint (mg/L)		Notes Numbered notes relate to general comments and/or MIC breakpoints.
	S ≤	R >	
Chloramphenicol	8	8	
Colistin	-	-	
Daptomycin	-	-	
Fosfomycin iv	-	-	
Fosfomycin oral	-	-	
Fusidic acid	-	-	
Metronidazole	4	4	
~~Mupirocin~~			
Nitrofurantoin (uncomplicated UTI only)	-	-	
Nitroxoline (uncomplicated UTI only)	-	-	
Rifampicin	-	-	
Spectinomycin	-	-	
Trimethoprim (uncomplicated UTI only)	-	-	
Trimethoprim-sulfamethoxazole	-	-	

Disk diffusion criteria for antimicrobial susceptibility testing of *Clostridium difficile* have not yet been defined and an MIC method should be used. If a commercial MIC method is used, follow the manufacturer´s instructions.

Fluoroquinolones	MIC breakpoint (mg/L)		Notes Numbered notes relate to general comments and/or MIC breakpoints.
	S ≤	R >	
Moxifloxacin	-[1]	-[1]	**1.** Not used clinically. May be tested for epidemiological purposes only (ECOFF 4 mg/L).

Glycopeptides	MIC breakpoint (mg/L)		Notes Numbered notes relate to general comments and/or MIC breakpoints.
	S ≤	R >	
Vancomycin	2[1]	2[1]	**1.** The breakpoints are based on epidemiological cut-off values (ECOFFs), which distinguish wild-type isolates from those with reduced susceptibility.

Tetracyclines	MIC breakpoint (mg/L)		Notes Numbered notes relate to general comments and/or MIC breakpoints.
	S ≤	R >	
Tigecycline	-[1,2]	-[1,2]	**1.** For tigecycline broth microdilution MIC determination, the medium must be prepared fresh on the day of use. **2.** Not used clinically. May be tested for epidemiological purposes only (ECOFF 0.25 mg/L).

Miscellaneous agents	MIC breakpoint (mg/L)		Notes Numbered notes relate to general comments and/or MIC breakpoints.
	S ≤	R >	
Daptomycin	-[1,2]	-[1,2]	**1.** Daptomycin MICs must be determined in the presence of Ca^{2+} (50 mg/L in the medium for broth dilution methods; agar dilution methods have not been validated). Follow the manufacturers' instructions for commercial systems. **2.** Not used clinically. May be tested for epidemiological purposes only (ECOFF 4 mg/L). **3.** Not used clinically. May be tested for epidemiological purposes only (ECOFF 2 mg/L). **4.** Fidaxomicin breakpoints and ECOFF have not been set because the available data show major variation in MIC distribution between studies. **5.** The breakpoints are based on epidemiological cut-off values (ECOFFs), which distinguish wild-type isolates from those with reduced susceptibility. **6.** Not used clinically. May be tested for epidemiological purposes only (ECOFF 0.004 mg/L).
Fusidic acid	-[3]	-[3]	
Fidaxomicin	IE[4]	IE[4]	
Metronidazole	2[5]	2[5]	
Rifampicin	-[6]	-[6]	

Gram-negative anaerobes

EUCAST Clinical Breakpoint Tables v. 7.1, valid from 2017-03-10

Disk diffusion criteria for antimicrobial susceptibility testing of anaerobes have not yet been defined and an MIC method should be used. If a commercial MIC method is used, follow the manufacturer´s instructions.

This group of bacteria includes many genera. The most frequently isolated Gram-negative anaerobes are *Bacteroides, Prevotella, Porphyromonas, Fusobacterium, Bilophila* and *Mobiluncus.* Anaerobes are most frequently defined by no growth on culture plates incubated in a CO_2 enriched atmosphere. For all these species, susceptibility testing should be performed in anaerobic environment.

Penicillins	MIC breakpoint (mg/L)		Notes Numbered notes relate to general comments and/or MIC breakpoints.
	S ≤	R >	
Benzylpenicillin[1]	0.25	0.5	**1.** Susceptibility to ampicillin, amoxicillin, piperacillin and ticarcillin can be inferred from susceptibility to benzylpenicillin.
Ampicillin[1]	0.5	2	**2.** For susceptibility testing purposes, the concentration of sulbactam is fixed at 4 mg/L.
Ampicillin-sulbactam	4[2]	8[2]	**3.** For susceptibility testing purposes, the concentration of clavulanic acid is fixed at 2 mg/L.
Amoxicillin[1]	0.5	2	**4.** For susceptibility testing purposes, the concentration of tazobactam is fixed at 4 mg/L.
Amoxicillin-clavulanic acid	4[3]	8[3]	
Piperacillin[1]	16	16	
Piperacillin-tazobactam	8[4]	16[4]	
Ticarcillin[1]	16	16	
Ticarcillin-clavulanic acid	8[3]	16[3]	
Temocillin	-	-	
Phenoxymethylpenicillin	IE	IE	
Oxacillin	-	-	
Cloxacillin	-	-	
Dicloxacillin	-	-	
Flucloxacillin	-	-	
Mecillinam (uncomplicated UTI only)	-	-	

Cephalosporins	MIC breakpoint (mg/L)		Notes Numbered notes relate to general comments and/or MIC breakpoints.
	S ≤	R >	
Cefaclor	-	-	
Cefadroxil	-	-	
Cefalexin	-	-	
Cefazolin	-	-	
Cefepime	-	-	
Cefixime	-	-	
Cefotaxime	-	-	
Cefoxitin	IE	IE	
Cefpodoxime	-	-	
Ceftaroline	-	-	
Ceftazidime	-	-	
Ceftazidime-avibactam	-	-	
Ceftibuten	-	-	
Ceftobiprole	-	-	
Ceftolozane-tazobactam	IE	IE	
Ceftriaxone	-	-	
Cefuroxime iv	-	-	
Cefuroxime oral	-	-	

Carbapenems	MIC breakpoint (mg/L)		Notes Numbered notes relate to general comments and/or MIC breakpoints.
	S ≤	R >	
Doripenem	1	1	
Ertapenem	1	1	
Imipenem	2	8	
Meropenem	2	8	

Monobactams	MIC breakpoint (mg/L)		Notes Numbered notes relate to general comments and/or MIC breakpoints.
	S ≤	R >	
Aztreonam	-	-	

Fluoroquinolones	MIC breakpoint (mg/L)		Notes
	S ≤	R >	Numbered notes relate to general comments and/or MIC breakpoints.
Ciprofloxacin	-	-	
Levofloxacin	-	-	
Moxifloxacin	IE	IE	
Nalidixic acid (screen)	NA	NA	
Norfloxacin (uncomplicated UTI only)	-	-	
Ofloxacin	-	-	

Aminoglycosides	MIC breakpoint (mg/L)		Notes
	S ≤	R >	Numbered notes relate to general comments and/or MIC breakpoints.
Amikacin	-	-	
Gentamicin	-	-	
Netilmicin	-	-	
Tobramycin	-	-	

Glycopeptides and lipoglycopeptides	MIC breakpoint (mg/L)		Notes
	S ≤	R >	Numbered notes relate to general comments and/or MIC breakpoints.
Dalbavancin	-	-	
Oritavancin	-	-	
Teicoplanin	-	-	
Telavancin	-	-	
Vancomycin	-	-	

Macrolides, lincosamides and streptogramins	MIC breakpoint (mg/L)		Notes
	S ≤	R >	Numbered notes relate to general comments and/or MIC breakpoints.
Azithromycin	-	-	
Clarithromycin	-	-	
Erythromycin	IE	IE	
Roxithromycin	-	-	
Telithromycin	-	-	
Clindamycin	4	4	
Quinupristin-dalfopristin	-	-	

Tetracyclines[1]	MIC breakpoint (mg/L)		Notes Numbered notes relate to general comments and/or MIC breakpoints.
	S ≤	R >	
Doxycycline	Note[1]	Note[1]	**1.** For anaerobic bacteria there is clinical evidence of activity in mixed intra-abdominal infections, but no correlation between MIC values, PK/PD data and clinical outcome. Therefore no breakpoints for susceptibility testing are given.
Minocycline	Note[1]	Note[1]	
Tetracycline	Note[1]	Note[1]	
Tigecycline	Note[1]	Note[1]	

Oxazolidinones	MIC breakpoint (mg/L)		Notes Numbered notes relate to general comments and/or MIC breakpoints.
	S ≤	R >	
Linezolid	-	-	
Tedizolid	-	-	

Miscellaneous agents	MIC breakpoint (mg/L)		Notes Numbered notes relate to general comments and/or MIC breakpoints.
	S ≤	R >	
Chloramphenicol	8	8	
Colistin	-	-	
Daptomycin	-	-	
Fosfomycin iv	-	-	
Fosfomycin oral	-	-	
Fusidic acid	-	-	
Metronidazole	4	4	
~~Mupirocin~~			
Nitrofurantoin (uncomplicated UTI only)	-	-	
Nitroxoline (uncomplicated UTI only)	-	-	
Rifampicin	-	-	
Spectinomycin	-	-	
Trimethoprim (uncomplicated UTI only)	-	-	
Trimethoprim-sulfamethoxazole	-	-	

Disk diffusion criteria for antimicrobial susceptibility testing of *Helicobacter pylori* have not yet been defined and an MIC method should be used. If a commercial MIC method is used, follow the manufacturer´s instructions.

Penicillins	MIC breakpoint (mg/L)		Notes Numbered notes relate to general comments and/or MIC breakpoints.
	S ≤	R >	
Amoxicillin	0.125[1]	0.125[1]	**1.** The breakpoints are based on epidemiological cut-off values (ECOFFs), which distinguish wild-type isolates from those with reduced susceptibility.

Fluoroquinolones	MIC breakpoint (mg/L)		Notes Numbered notes relate to general comments and/or MIC breakpoints.
	S ≤	R >	
Levofloxacin	1[1]	1[1]	**1.** The breakpoints are based on epidemiological cut-off values (ECOFFs), which distinguish wild-type isolates from those with reduced susceptibility.

Macrolides	MIC breakpoint (mg/L)		Notes Numbered notes relate to general comments and/or MIC breakpoints.
	S ≤	R >	
Clarithromycin	0.25[1]	0.5[1]	**1.** The breakpoints are based on epidemiological cut-off values (ECOFFs), which distinguish wild-type isolates from those with reduced susceptibility.

Tetracyclines	MIC breakpoint (mg/L)		Notes Numbered notes relate to general comments and/or MIC breakpoints.
	S ≤	R >	
Tetracycline	1[1]	1[1]	**1.** The breakpoints are based on epidemiological cut-off values (ECOFFs), which distinguish wild-type isolates from those with reduced susceptibility.

Miscellaneous agents	MIC breakpoint (mg/L)		Notes Numbered notes relate to general comments and/or MIC breakpoints.
	S ≤	R >	
Metronidazole	8[1]	8[1]	**1.** The breakpoints are based on epidemiological cut-off values (ECOFFs), which distinguish wild-type isolates from those with reduced susceptibility.
Rifampicin	1[1]	1[1]	

Disk diffusion (EUCAST standardised disk diffusion method)
Medium: Mueller-Hinton agar + 5% defibrinated horse blood and 20 mg/L β-NAD (MH-F)
Inoculum: McFarland 0.5
Incubation: 5% CO_2, 35±1ºC, 18±2h
Reading: Read zone edges as the point showing no growth viewed from the front of the plate with the lid removed and with reflected light.
Quality control: *Streptococcus pneumoniae* ATCC 49619

Penicillins	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Benzylpenicillin	1	1	1 unit	13	13	
Ampicillin	1	1	2	16	16	

Carbapenems	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Meropenem	0.25	0.25	10	26	26	

Macrolides	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Erythromycin	1	1	15	25	25	

Miscellaneous agents	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Trimethoprim-sulfamethoxazole[1]	0.06	0.06	1.25-23.75	29	29	**1.** Trimethoprim-sulfamethoxazole in the ratio 1:19. Breakpoints are expressed as the trimethoprim concentration.

Disk diffusion (EUCAST standardised disk diffusion method)
Medium: Mueller-Hinton agar + 5% defibrinated horse blood and 20 mg/L β-NAD (MH-F)
Inoculum: McFarland 0.5
Incubation: 5% CO_2, 35±1ºC, 18±2h
Reading: Read zone edges as the point showing no growth viewed from the front of the plate with the lid removed and with reflected light.
Quality control: *Haemophilus influenzae* ATCC 49766. For control of the inhibitor component of beta-lactam inhibitor-combination disks, use *Staphylococcus aureus* ATCC 29213.

Penicillins	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Benzylpenicillin	0.5	0.5	1 unit	17	17	**1.** For susceptibility testing purposes, the concentration of clavulanic acid is fixed at 2 mg/L.
Ampicillin	1	1		Note[A]	Note[A]	
Amoxicillin	1	1		Note[A]	Note[A]	**A.** Infer susceptibility from benzylpenicillin susceptibility.
Amoxicillin-clavulanic acid	1[1]	1[1]	2-1	15	15	

Cephalosporins	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Cefotaxime	0.03	0.03	5	26	26	

Fluoroquinolones	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Ciprofloxacin	0.06	0.06	5	27[A]	27[A]	**A.** The nalidixic acid disk diffusion test can be used to screen for fluoroquinolone resistance. **See Note B.**
Levofloxacin	0.06	0.06	5	27[A]	27[A]	**B.** Isolates categorised as susceptible to nalidixic acid can be reported susceptible to ciprofloxacin and levofloxacin. Isolates categorised as non-susceptible may have fluoroquinolone resistance and should be tested against the appropriate agent.
Nalidixic acid (screen)	NA	NA	30	23[B]	Note[B]	

Tetracyclines	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Doxycycline	1	1		Note[A]	Note[A]	**A.** Susceptibility inferred from tetracycline screen test.
Tetracycline (screen)	NA	NA	30	24[A]	24[A]	

Miscellaneous agents	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Trimethoprim-sulfamethoxazole[1]	0.25	0.25	1.25-23.75	23	23	**1.** Trimethoprim-sulfamethoxazole in the ratio 1:19. Breakpoints are expressed as the trimethoprim concentration.

Disk diffusion (EUCAST standardised disk diffusion method)
Medium: Mueller-Hinton agar + 5% defibrinated horse blood and 20 mg/L β-NAD (MH-F). The MH-F plates should be dried prior to inoculation to reduce swarming (at 20-25°C overnight or at 35°C, with the lid removed, for 15 min).
Inoculum: McFarland 0.5
Incubation: Microaerobic environment, 41±1ºC, 24h. Isolates with insufficient growth after 24h incubation are reincubated immediately and inhibition zones read after a total of 40-48h incubation.
Reading: Read zone edges as the point showing no growth viewed from the front of the plate with the lid removed and with reflected light.
Quality control: *Campylobacter jejuni* ATCC 33560

Fluoroquinolones	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Ciprofloxacin	0.5	0.5	**5**	26	26	

Macrolides	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Azithromycin	Note[1]	Note[1]		Note[A]	Note[A]	**1/A.** Erythromycin can be used to determine susceptibility to azithromycin and clarithromycin.
Clarithromycin	Note[1]	Note[1]		Note[A]	Note[A]	
Erythromycin, *C. jejuni*	4[1]	4[1]	**15**	20[A]	20[A]	
Erythromycin, *C. coli*	8[1]	8[1]	**15**	24[A]	24[A]	

Tetracyclines	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Doxycycline	Note[1]	Note[1]		Note[A]	Note[A]	**1/A.** Tetracycline can be used to determine susceptibility to doxycycline.
Tetracycline	2[1]	2[1]	**30**	30[A]	30[A]	

Disk diffusion (EUCAST standardised disk diffusion method)
Medium: Mueller-Hinton agar + 5% defibrinated horse blood and 20 mg/L β-NAD (MH-F)
Inoculum: McFarland 0.5
Incubation: 5% CO_2, 35±1ºC, 18±2h. Isolates with insufficient growth after 16-20h incubation are reincubated immediately and inhibition zones read after a total of 40-44h incubation.
Reading: Read zone edges as the point showing no growth viewed from the front of the plate with the lid removed and with reflected light.
Quality control: *Streptococcus pneumoniae* ATCC 49619

Penicillins	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Benzylpenicillin	0.125	0.125	1 unit	29	29	

Fluoroquinolones	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Ciprofloxacin	1	1	5	25	25	
Moxifloxacin	0.5	0.5	5	25	25	

Aminoglycosides	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Gentamicin	1	1	10	23	23	

Glycopeptides	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Vancomycin	2	2	5	17	17	

Lincosamides	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Clindamycin	0.5	0.5	2	20	20	

Tetracyclines	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Tetracycline	2	2	30	24	24	

Oxazolidinones	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Linezolid	2	2	10	25	25	

Miscellaneous agents	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Rifampicin	0.06	0.5	5	30	25	

Disk diffusion (EUCAST standardised disk diffusion method)
Medium: Mueller-Hinton agar + 5% defibrinated horse blood and 20 mg/L β-NAD (MH-F)
Inoculum: McFarland 0.5
Incubation: 5% CO_2, 35±1ºC, 18±2h. Isolates with insufficient growth after 16-20h incubation are reincubated immediately and inhibition zones read after a total of 40-44h incubation.
Reading: Read zone edges as the point showing no growth viewed from the front of the plate with the lid removed and with reflected light.
Quality control: *Streptococcus pneumoniae* ATCC 49619

Penicillins	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Benzylpenicillin	0.125	0.125	1 unit	21	21	**1/A.** Infer susceptibility from ampicillin susceptibility.
Ampicillin	0.25	0.25	2	26	26	
Amoxicillin	Note[1]	Note[1]		Note[A]	Note[A]	

Carbapenems	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Meropenem	0.25	0.25	10	31	31	

Fluoroquinolones	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Ciprofloxacin (uncomplicated UTI only)	2	2	5	21[A]	21[A]	**1.** Susceptibility can be inferred from ciprofloxacin susceptibility.
Levofloxacin (uncomplicated UTI only)	2[1]	2[1]	5	Note[B]	Note[B]	**A.** Susceptibility can be inferred from norfloxacin susceptibility. **See Note C.**
Norfloxacin (screen)	NA	NA	10	17[C]	17[C]	**B.** Susceptibility can be inferred from ciprofloxacin or norfloxacin susceptibility. **See Note C.** **C.** The norfloxacin disk diffusion test can be used to screen for fluoroquinolone resistance.

Glycopeptides	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Vancomycin	1	1	5	16	16	

Aerococcus sanguinicola and *urinae*

EUCAST Clinical Breakpoint Tables v. 7.1, valid from 2017-03-10

Miscellaneous agents	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Nitrofurantoin (uncomplicated UTI only)	16	16	100	16	16	
Rifampicin	0.125	0.125	5	25	25	

Kingella kingae

EUCAST Clinical Breakpoint Tables v. 7.1, valid from 2017-03-10

Disk diffusion (EUCAST standardised disk diffusion method)
Medium: Mueller-Hinton agar + 5% defibrinated horse blood and 20 mg/L β-NAD (MH-F)
Inoculum: McFarland 0.5
Incubation: 5% CO_2, 35±1ºC, 18±2h. Isolates with insufficient growth after 16-20h incubation are reincubated immediately and inhibition zones read after a total of 40-44h incubation.
Reading: Read zone edges as the point showing no growth viewed from the front of the plate with the lid removed and with reflected light.
Quality control: *Haemophilus influenzae* ATCC 49766.

Penicillins[1]	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Benzylpenicillin	0.03	0.03	1 unit	25	25	**1.** Beta-lactamase positive isolates can be reported resistant to ampicillin and amoxicillin without inhibitors. Tests based on a chromogenic cephalosporin can be used to detect the beta-lactamase. Beta-lactam resistance mechanisms other than beta-lactamase production have not yet been described for *K. kingae* . **2.** Susceptibility can be inferred from benzylpenicillin susceptibility. **3/B.** The intrinsic activity of clavulanic acid in *K. kingae* is such that the organism is inhibited by ≤2 mg/L clavulanic acid. Therefore no MIC breakpoints for amoxicillin-clavulanic acid can be given. **A.** Infer susceptibility from benzylpenicillin susceptibility.
Ampicillin	0.06[2]	0.06[2]		Note[A]	Note[A]	
Amoxicillin	0.125[2]	0.125[2]		Note[A]	Note[A]	
Amoxicillin-clavulanic acid	Note[3]	Note[3]		Note[B]	Note[B]	

Cephalosporins	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Cefotaxime	0.125	0.125	5	27	27	
Ceftriaxone	0.06	0.06	30	30	30	
Cefuroxime iv	0.5	0.5	30	29	29	

Carbapenems	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Meropenem	0.03	0.03	10	30	30	

Fluoroquinolones	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Ciprofloxacin	0.06	0.06	5	28	28	
Levofloxacin	0.125	0.125	5	28	28	

Macrolides and lincosamides	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Azithromycin	0.25[1]	0.25[1]		Note[A]	Note[A]	**1.** Susceptibility can be inferred from erythromycin susceptibility.
Clarithromycin	0.5[1]	0.5[1]		Note[A]	Note[A]	**A.** Infer susceptibility from erythromycin susceptibility.
Erythromycin	0.5	0.5	**15**	20	20	
Clindamycin	-	-		-	-	

Tetracyclines	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Doxycycline	0.5[1]	0.5[1]		Note[A]	Note[A]	**1/A.** Isolates susceptible to tetracycline are also susceptible to doxycycline, but some resistant to tetracycline may be susceptible to doxycycline. An MIC method should be used to test doxycycline susceptibility of tetracycline resistant isolates if required.
Tetracycline	0.5	0.5	**30**	28	28	

Miscellaneous agents	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Rifampicin	0.5	0.5	**5**	20	20	**1.** Trimethoprim:sulfamethoxazole in the ratio 1:19. Breakpoints are expressed as the trimethoprim concentration.
Trimethoprim-sulfamethoxazole[1]	0.25	0.25	**1.25-23.75**	28	28	

Mycobacterium tuberculosis

EUCAST Clinical Breakpoint Tables v. 7.1, valid from 2017-03-10

Listed breakpoints have been set in parallel with marketing authorisation by EMA. Breakpoints for other agents have not yet been established.

Recommended methods for antimicrobial susceptibility testing of mycobacteria are currently under discussion.

	MIC breakpoint (mg/L)		Notes Numbered notes relate to general comments and/or MIC breakpoints.
	S ≤	R >	
Delamanid	0.06	0.06	
Bedaquiline	0.25	0.25	

ECOFFs and systemic clinical breakpoints for antimicrobial agents that are used topically

In the absence of clinical data on outcome related to MIC of infecting organisms EUCAST does not find it possible to reach a consensus that resolves the conflicting opinions on these two alternative proposals (**for details see guidance document**):
1. Use ECOFFs for all agents when used topically.
2. Use clinical breakpoints when available and ECOFFs when there are no clinical breakpoints.

For information, the table presents systemic clinical breakpoints and ECOFFs for agents that are used both systemically and topically, and ECOFFs for agents that are used topically only (note that the mupirocin breakpoints are the exception).

Organisms		Gentamicin[3]	Ciprofloxacin[3]	Levofloxacin[3]	Ofloxacin[3]	Chloramphenicol[3]	Colistin[3] (for Polymyxin B)	Fusidic acid[3]	Neomycin (framycetin)	Bacitracin	Mupirocin	Retapamulin
Enterobacteriaceae	**ECOFF**[1,2]	2	0.125	0.25	0.5	16	2	-	8	-	-	-
	Systemic clinical breakpoint[1]	2/4	0.25/0.5	0.5/1	0.25/0.5	8/8	2/2	-	-	-	-	-
P. aeruginosa	**ECOFF**[1]	8	0.5	2	2	-	4	-	ND	-	-	-
	Systemic clinical breakpoint[1]	4/4	0.5/0.5	1/1	-	-	2/2	-	-	-	-	-
***Acinetobacter* spp.**	**ECOFF**[1,2]	4	1	0.5	1	-	2	-	ND	-	-	-
	Systemic clinical breakpoint[1]	4/4	1/1	0.5/1	-	-	2/2	-	-	-	-	-
S. aureus	**ECOFF**[1]	2	1	1	1	16	-	0.5	1	ND	1[4]	0.5
	Systemic clinical breakpoint[1]	1/1	1/1	1/1	1/1	8/8	-	1/1	-	-	-	-
S. pneumoniae	**ECOFF**[1]	-	2	2	4	8	-	32	ND	ND	-	-
	Systemic clinical breakpoint[1]	-	-	2/2	-	8/8	-	-	-	-	-	-
Streptococcus A, B, C and G	**ECOFF**[1,2]	-	2	2	4	8	-	32	ND	ND	0.5	0.125
	Systemic clinical breakpoint[1]	-	-	2/2	-	8/8	-	IE	-	-	-	-
H. influenzae	**ECOFF**[1]	4	0.06	0.06	0.125	1	-	ND	ND	-	-	-
	Systemic clinical breakpoint[1]	IE	0.06/0.06	0.06/0.06	0.06/0.06	2/2	-	-	-	-	-	-
***Moraxella* spp.**	**ECOFF**[1,2]	0.25	0.125	0.125	0.25	2	-	ND	ND	-	-	-
	Systemic clinical breakpoint[1]	IE	0.5/0.5	1/1	0.5/0.5	2/2	-	-	-	-	-	-

Notes

[1] ECOFFs and systemic clinical breakpoints in mg/L.

[2] This ECOFF is representative of ECOFFs for the most relevant species.

[3] Agents also available for systemic use.

[4] Breakpoints for nasal decontamination S≤1, R>256 mg/L (S≥30, R<18 mm for the mupirocin 200 µg disks). Intermediate isolates are associated with short term suppression (useful preoperatively) but, unlike susceptible isolates, long term eradication rates are low.

ND = No ECOFF defined on EUCAST MIC distribution website.

PK/PD (Non-species related) breakpoints

EUCAST Clinical Breakpoint Tables v. 7.1, valid from 2017-03-10

These breakpoints are used only when there are no species-specific breakpoints or other recommendations (a dash or a note) in the species-specific tables.

Penicillins	MIC breakpoint (mg/L)		Notes
	S ≤	R >	
Benzylpenicillin	0.25	2	**1.** For susceptibility testing purposes, the concentration of sulbactam is fixed at 4 mg/L.
Ampicillin	2	8	**2.** For susceptibility testing purposes, the concentration of clavulanic acid is fixed at 2 mg/L.
Ampicillin-sulbactam	2[1]	8[1]	**3.** For susceptibility testing purposes, the concentration of tazobactam is fixed at 4 mg/L.
Amoxicillin	2	8	
Amoxicillin-clavulanic acid	2[2]	8[2]	
Piperacillin	4	16	
Piperacillin-tazobactam	4[3]	16[3]	
Ticarcillin	8	16	
Ticarcillin-clavulanic acid	8[2]	16[2]	
Temocillin	IE	IE	
Phenoxymethylpenicillin	IE	IE	
Oxacillin	IE	IE	
Cloxacillin	IE	IE	
Dicloxacillin	IE	IE	
Flucloxacillin	IE	IE	
Mecillinam	IE	IE	

Cephalosporins	MIC breakpoint (mg/L)		Notes
	S ≤	R >	
Cefaclor	IE	IE	**1**. Based on PK/PD target for Gram-negative organisms. **2**. Breakpoints are based on ceftolozane data. **3**. For susceptibility testing purposes, the concentration of tazobactam is fixed at 4 mg/L. **4**. For susceptibility testing purposes, the concentration of avibactam is fixed at 4 mg/L.
Cefadroxil	IE	IE	
Cefalexin	IE	IE	
Cefazolin	1	2	
Cefepime	4	8	
Cefixime	IE	IE	
Cefotaxime	1	2	
Cefoxitin	IE	IE	
Cefpodoxime	IE	IE	
Ceftaroline	0.5[1]	0.5[1]	
Ceftazidime	4	8	
Ceftazidime-avibactam	8[4]	8[4]	
Ceftibuten	IE	IE	
Ceftobiprole	4	4	
Ceftolozane-tazobactam	4[2,3]	4[2,3]	
Ceftriaxone	1	2	
Cefuroxime iv	4	8	
Cefuroxime oral	IE	IE	

Carbapenems	MIC breakpoint (mg/L)		Notes
	S ≤	R >	
Doripenem	1	2	
Ertapenem	0.5	1	
Imipenem	2	8	
Meropenem	2	8	

Monobactams	MIC breakpoint (mg/L)		Notes
	S ≤	R >	
Aztreonam	4	8	

Fluoroquinolones	MIC breakpoint (mg/L)		Notes
	S ≤	R >	
Ciprofloxacin	0.25	0.5	
Levofloxacin	0.5	1	
Moxifloxacin	0.25	0.25	
Nalidixic acid (screen)	IE	IE	
Norfloxacin	IE	IE	
Ofloxacin	0.25	0.5	

Aminoglycosides	MIC breakpoint (mg/L)		Notes
	S ≤	R >	
Amikacin	IE	IE	
Gentamicin	IE	IE	
Netilmicin	IE	IE	
Tobramycin	IE	IE	

Glycopeptides and lipoglycopeptides	MIC breakpoint (mg/L)		Notes
	S ≤	R >	
Dalbavancin	0.25[1]	0.25[1]	**1.** For broth microdilution MIC determination, the medium must be supplemented with polysorbate-80 to a final concentration of 0.002%.
Oritavancin	0.125[1,2]	0.125[1,2]	**2.** PK/PD breakpoints are based on *S. aureus* . For *S. pyogenes* there is uncertainty regarding the PK/PD target. For broth microdilution MIC determination, the medium must be supplemented with polysorbate-80 to a final concentration of 0.002%.
Teicoplanin	IE	IE	
Telavancin	IE	IE	
Vancomycin	IE	IE	

Macrolides, lincosamides and streptogramins	MIC breakpoint (mg/L)		Notes
	S ≤	R >	
Azithromycin	IE	IE	
Clarithromycin	IE	IE	
Erythromycin	IE	IE	
Roxithromycin	IE	IE	
Telithromycin	IE	IE	
Clindamycin	IE	IE	
Quinupristin-dalfopristin	IE	IE	

Tetracyclines	MIC breakpoint (mg/L)		Notes
	S ≤	R >	
Doxycycline	IE	IE	**1.** For tigecycline broth microdilution MIC determination, the medium must be prepared fresh on the day of use.
Minocycline	IE	IE	
Tetracycline	IE	IE	
Tigecycline	0.25[1]	0.5[1]	

Oxazolidinones	MIC breakpoint (mg/L)		Notes
	S ≤	R >	
Linezolid	2	4	
Tedizolid	IE	IE	

Miscellaneous agents	MIC breakpoint (mg/L)		Notes
	S ≤	R >	
Chloramphenicol	IE	IE	
Colistin	IE	IE	
Daptomycin	IE	IE	
Fosfomycin iv	IE	IE	
Fosfomycin oral	IE	IE	
Fusidic acid	IE	IE	
Metronidazole	IE	IE	
~~Mupirocin~~			
Nitrofurantoin	IE	IE	
Nitroxoline	IE	IE	
Rifampicin	IE	IE	
Spectinomycin	IE	IE	
Trimethoprim	IE	IE	
Trimethoprim-sulfamethoxazole	IE	IE	

Dosages

EUCAST breakpoints are based on the following dosages (see section 8 in Rationale Documents).

Penicillins	Standard dose	High dose
Benzylpenicillin	0.6 g x 4 iv	2.4 g x 6 iv
Ampicillin	0.5 -1 g x 3-4 iv	1-2 g x 4-6 iv
Ampicillin-sulbactam	3 g x 3 iv	4 g x 3 iv
Amoxicillin	0.5 g x 3 iv Oral dosage under discussion	2 g x 6 iv Oral dosage under discussion
Amoxicillin-clavulanic acid	(1 g amoxicillin + 0.2 g clavulanic acid) x 3 iv Oral dosage under discussion	(2 g amoxicillin + 0.2 g clavulanic acid) x 3 iv Oral dosage under discussion
Piperacillin	4 g x 3 iv	4 g x 4 iv
Piperacillin-tazobactam	(4 g piperacillin + 0.5 g tazobactam) x 3 iv	(4 g piperacillin + 0.5 g tazobactam) x 4 iv
Ticarcillin	3 g x 4 iv	3 g x 6 iv
Ticarcillin-clavulanic acid	(3 g ticarcillin + 0.1 g clavulanic acid) x 4 iv	(3 g ticarcillin + 0.1 g clavulanic acid) x 6 iv
Temocillin		
Phenoxymethylpenicillin	0.5-2 g x 3-4	None
Oxacillin	Clinical breakpoints not available	Clinical breakpoints not available
Cloxacillin	0.5 g x 4 oral or 1 g x 4 iv	1 g x 4 oral or 2 g x 6 iv
Dicloxacillin	0.5-1 g x 4 oral or 1 g x 4 iv	2 g x 4 oral or 2 g x 6 iv
Flucloxacillin	1 g x 3 oral or 2 g x 4 iv	1 g x 4 oral or 2 g x 6 iv
Mecillinam	0.2-0.4 g x 3 oral	None

Cephalosporins	Standard dose	High dose
Cefaclor	0.25-1 g x 3 oral	None
Cefadroxil	0.5-1 g x 2 oral	None
Cefalexin	0.25-1 g x 2-3 oral	None
Cefazolin	1-2 g x 3	None
Cefepime	2 g x 2 iv	2 g x 3 iv
Cefixime	0.2-0.4 g x 2 (0.4 g as single dose for *Neisseria gonorrhoeae*)	None
Cefotaxime	1 g x 3 iv	2 g x 3 iv
Cefoxitin	Clinical breakpoints not available	Clinical breakpoints not available
Cefpodoxime	0.1-0.2 g x 2 oral	None
Ceftaroline	0.6 g x 2 iv over 1 hour	0.6 g x 3 iv over 2 hours
Ceftazidime	1 g x 3 iv	2 g x 3 iv
Ceftazidime-avibactam	(2 g ceftazidime + 0.5 g avibactam) x 3 over 2 hours	
Ceftibuten	0.4 g x 1 oral	None
Ceftobiprole	0.5 g x 3 iv over 2 hours	None
Ceftolozane-tazobactam	(1 g ceftolozane + 0.5 g tazobactam) x 3 iv over 1 hour	None
Ceftriaxone	1 g x 1 iv	2 g x 1 iv
Cefuroxime iv	0.75 g x 3 iv	1.5 g x 3 iv
Cefuroxime oral	0.25-0.5 g x 2 oral	None

Carbapenems	Standard dose	High dose
Doripenem	0.5 g x 3 iv over 1 hour	1 g x 3 iv over 4 hours
Ertapenem	1 g x 1 iv over 30 minutes	None
Imipenem	0.5 g x 4 iv over 30 minutes	1 g x 4 iv over 30 minutes
Meropenem	1 g x 3 iv over 30 minutes	2 g x 3 iv over 30 minutes

Monobactams	Standard dose	High dose
Aztreonam	1 g x 3 iv	2 g x 4 iv

Fluoroquinolones	Standard dose	High dose
Ciprofloxacin	0.5 g x 2 oral or 0.4 g x 2 iv	0.75 g x 2 oral or 0.4 g x 3 iv
Levofloxacin	0.5 g x 1 oral or 0.5 g x 1 iv	0.5 g x 2 oral or 0.5 g x 2 iv
Moxifloxacin	0.4 g x 1 oral or 0.4 g x 1 iv	None
Nalidixic acid	Laboratory test reagent only	Laboratory test reagent only
Norfloxacin	0.4 g x 2 oral	None
Ofloxacin	0.2 g x 2 oral or 0.2 g x 2 iv	0.4 g x 2 oral or 0.4 g x 2 iv

Aminoglycosides	Standard dose	High dose
Amikacin	20 mg/kg x 1 iv	30 mg/kg x 1 iv
Gentamicin	5 mg/kg x 1 iv	7 mg/kg x 1 iv
Netilmicin	5 mg/kg x 1 iv	7 mg/kg x 1 iv
Tobramycin	5 mg/kg x 1 iv	7 mg/kg x 1 iv

Glycopeptides and lipoglycopeptides	Standard dose	High dose
Dalbavancin	1 g x 1 iv over 30 minutes on day 1 If needed, 0.5 g x 1 iv over 30 minutes on day 8	None
Oritavancin	1.2 g x 1 (single dose) iv over 3 hours	None
Teicoplanin	0.4 g x 1 iv	0.8 g x 1 iv or 0.4 g x 2 iv
Telavancin	10 mg/kg x 1 iv over 1 hour	None
Vancomycin	0.5 g x 4 iv or 1 g x 2 iv or 2 g x 1 by continuous infusion	None

Macrolides, lincosamides and streptogramins	Standard dose	High dose
Azithromycin	0.5 g x 1 oral or 0.5 g x 1 iv (2 g as single dose for *Neisseria gonorrhoeae*)	None
Clarithromycin	0.25 g x 2 oral	0.5 g x 2 oral
Erythromycin	0.5 g x 2-4 oral or 0.5 g x 2-4 iv	1 g x 4 oral or 1 g x 4 iv
Roxithromycin	0.15 g x 2 oral	None
Telithromycin	0.8 g x 1 oral	None
Clindamycin	0.3 g x 2 oral or 0.6 g x 3 iv	0.3 g x 4 oral or 1.2 g x 2 iv
Quinupristin-dalfopristin	7.5 mg/kg x 2	7.5 mg/kg x 3

Tetracyclines	Standard dose	High dose
Doxycycline	0.1 g x 1 oral	0.2 g x 1 oral
Minocycline	0.1 g x 2 oral	None
Tetracycline	0.25 g x 4 oral	0.5 g x 4 oral
Tigecycline	0.1 g loading dose followed by 50 mg x 2 iv	None

Oxazolidinones	Standard dose	High dose
Linezolid	0.6 g x 2 oral or 0.6 g x 2 iv	None
Tedizolid	0.2 g x 1 oral	None

Miscellaneous agents	Standard dose	High dose
Chloramphenicol	1 g x 4 oral or 1 g x 4 iv	2 g x 4 oral or 2 g x 4 iv
Colistin	3 MU x 3 iv with a loading dose of 9 MU	None
Daptomycin	0.25 g x 1 iv	0.5 g x 1 iv
Fosfomycin iv	4 g x 3 iv	8 g x 3 iv
Fosfomycin oral	3 g x 1 oral as a single dose	None
Fusidic acid	0.5 g x 2 oral or 0.5 g x 2 iv	0.5 g x 3 oral or 0.5 g x 3 iv
Metronidazole	0.4 g x 3 oral or 0.4 g x 3 iv	0.5 g x 3 oral or 0.5 g x 3 iv
~~Mupirocin~~		
Nitrofurantoin	50 mg x 3 oral	0.1 g x 4 oral
Nitroxoline	0.25 g x 3	None
Rifampicin	0.6 g x 1 oral or 0.6 g x 1 iv	0.6 g x 2 oral or 0.6 g x 2 iv
Spectinomycin	2 g x 1 im	None
Trimethoprim	0.16 g x 2 oral	None
Trimethoprim-sulfamethoxazole	(0.16 g trimethoprim + 0.8 g sulfa) x 2 oral or (0.16 g trimethoprim + 0.8 g sulfa) x 2 iv	(0.24 g trimethoprim + 1.2 g sulfa) x 2 oral or (0.24 g trimethoprim + 1.2 g sulfa) x 2 iv