World Health Organization
REGIONAL OFFICE FOR **Europe**

Central Asian and European Surveillance of Antimicrobial Resistance

CAESAR Manual

Version 3.0 2019

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and European
Surveillance of
**Antimicrobial
Resistance**

*CAESAR Manual
Version 3, 2019*

Abstract

This manual is an update of the first edition published in 2015 and describes the objectives, methods and organization of the Central Asian and European Surveillance of Antimicrobial Resistance (CAESAR) network. It details steps involved for a country or area wanting to enrol in CAESAR, as well as steps involved in routine data collection for antimicrobial resistance (AMR) surveillance. It contains the protocols and AMR case definitions used by the network. Key updates involve the addition of Salmonella species to the list of pathogens under surveillance, as well as updates related to the European Committee on Antimicrobial Susceptibility Testing categories. CAESAR continues to coordinate closely with the European Antimicrobial Resistance Surveillance Network (EARS-Net) and strives for compatibility and comparability with EARS-NET, as well as the Global AMR Surveillance System coordinated by WHO headquarters.

Keywords

Contents

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Abbreviations

AMR	antimicrobial resistance
AST	antimicrobial susceptibility testing
ß-NAD	ß-nicotinamide adenine dinucleotide
CAESAR	Central Asian and European Surveillance of Antimicrobial Resistance
CLSI	Clinical and Laboratory Standards Institute
CO_2	carbon dioxide
CSF	cerebrospinal fluid
CSV file	comma-separated values file
E. coli	*Escherichia coli*
E. faecalis	*Enterococcus faecalis*
E. faecium	*Enterococcus faecium*
EARS-Net	European Antimicrobial Resistance Surveillance Network
ECDC	European Centre for Disease Prevention and Control
eIRF	electronic isolate record form
EQA	external quality assessment
ESBL	extended-spectrum ß-lactamase
ESCMID	European Society of Clinical Microbiology and Infectious Diseases
EUCAST	European Committee on Antimicrobial Susceptibility Testing
GLASS	Global Antimicrobial Resistance Surveillance System
ICU	intensive care unit
IRF	isolate record form
ISO	International Standards Organization
K. pneumoniae	*Klebsiella pneumoniae*
LIS	laboratory information system
MH	Mueller-Hinton
MH-F	Mueller-Hinton Fastidious
MIC	minimum inhibitory concentration

MRSA	methicillin-resistant *Staphylococcus aureus*
NRL	national AMR reference laboratory
P. aeruginosa	*Pseudomonas aeruginosa*
PBP2a	penicillin-binding protein 2a
PoP project	Proof-of-Principle routine diagnostic project for antimicrobial resistance surveillance
QC	quality control
RIVM	National Institute for Public Health and the Environment of the Netherlands
S. aureus	*Staphylococcus aureus*
S. pneumoniae	*Streptococcus pneumoniae*
spp.	species (for specific bacteria)
susceptibility category (S/I/R)	susceptibility of a pathogen to an antimicrobial agent according to clinical breakpoints
	S = susceptible, standard dosing regimen
	I = susceptible, increased exposure
	R = resistant
TESSy	The European Surveillance System (ECDC)
UTI	urinary tract infection
WHONET	WHO microbiology laboratory database software

Scope and purpose

This manual describes the objectives, methodology, and organization of the Central Asian and European Surveillance of Antimicrobial Resistance (CAESAR) network. The purpose of this document is to provide practical and technical support for countries and areas striving to participate in the CAESAR network.

This manual is divided into six chapters. Chapters 1 and 2 introduce the scope and objectives of the CAESAR network and provide an overview of the CAESAR methodology. Chapter 3 describes the steps to be taken by a country planning to set up a national antimicrobial resistance surveillance system. Chapters 4–6 contain information about how to collect and handle antimicrobial susceptibility testing data, how to prepare the datasets for submission to CAESAR, and how data are analysed within CAESAR.

In addition, this manual contains annexes on useful tools for collecting information on laboratory and hospital characteristics (Annex A), laboratory methods (Annex B), forms and manuals to make data collection easier and more reliable (Annexes C and D), a list of CAESAR variables (Annex E), and an overview of pathogens under CAESAR surveillance (Annex F).

The third edition of the CAESAR manual builds on the second edition published in 2015. The manual is adapted from the European Centre for Disease Prevention and Control (ECDC) European Antimicrobial Resistance Surveillance Network (EARS-Net) reporting protocol (latest version 2018) and the microbiological manual from the European Antimicrobial Resistance Surveillance System (EARSS), the predecessor of EARS-Net (version 2005). In an effort to align CAESAR methodology with that of the Global Antimicrobial Resistance Surveillance System (GLASS), in 2016 *Salmonella* was added to the list of pathogens under CAESAR surveillance.

CHAPTER

1

Introduction

1.1 Background

The discovery of antibiotics and other antimicrobial agents has dramatically changed human and veterinary medicine, preventing and curing infections and saving millions of lives. However, bacteria and other microorganisms become resistant to antimicrobial treatment through the natural process of adaptation. The overuse and misuse of antimicrobial agents, poor hygiene, and the lack of infection prevention measures greatly accelerate the rate at which this resistance emerges. Resistance that develops in one organism or location can spread to other organisms or locations and can consequently affect antimicrobial treatment of a wide range of infections and diseases. Reduced effectiveness of antimicrobial agents leads to longer illnesses, prolonged stays in hospitals, increased mortality, and reduced protection of patients undergoing surgery or chemotherapy. The problem of antimicrobial resistance (AMR) is exacerbated by the fact that very few new antimicrobial drugs are being developed. AMR is a global problem and the WHO European Region is no exception; several countries now report resistance rates of some pathogens against commonly used antibiotics of more than 50% *(1,2)* and resistance mechanisms are emerging and spreading rapidly.

Without harmonized and immediate action on a global scale, the world is heading towards a post-antibiotic era in which common infections that currently can be effectively treated with antimicrobial treatment may once again result in death *(3)*. In response to this crisis, all 53 Member States in the WHO European Region adopted the European Strategic Action Plan on Antibiotic Resistance in September 2011, in Baku, Azerbaijan, at the 61st session of the WHO Regional Committee for Europe. This Action Plan aims to preserve the ability of modern medicine to prevent and treat infections for the current and future generations *(4)*. In 2015, the World Health Assembly adopted the global action plan on antimicrobial resistance. The goal of the global action plan is to ensure, for as long as possible, continuity of successful treatment and prevention of infectious diseases with effective and safe medicines that are quality-assured, used in a responsible way, and accessible to all who need them. More recently, the growing awareness among national leaders was demonstrated by the United Nations General Assembly High-Level Meeting on Antimicrobial Resistance in September 2016, which was only the fourth time the General Assembly has held a high-level meeting for a health issue. In a political declaration, all 193 United Nations Member States committed themselves to develop multisectoral national action plans in line with the World Health Assembly's global action plan on antimicrobial resistance, including its second strategic objective: to strengthen the knowledge and evidence base through surveillance and research.

One of the main pillars of the European and global action plans is the implementation of systematic surveillance. AMR surveillance is the systematic, ongoing process of data collection, analysis, and reporting that quantitatively monitors temporal trends in the occurrence and distribution of susceptibility and resistance to antimicrobial agents. AMR surveillance provides essential information needed to guide medical practice, including therapeutics and disease control activities *(5)*. Antimicrobial susceptibility information contributes to improving the quality and safety and lowering the costs of health care, for example by guiding empiric therapy and supporting infection prevention and control and antibiotic stewardship programmes. Appropriate and targeted use of antimicrobials improves patient outcomes and reduces the rate at which resistance emerges and spreads. Regular analysis and feedback of surveillance data to health care workers allow the timely adjustment of antimicrobial therapy guidelines and initiation of measures to control hospital and community outbreaks of multidrug-resistant bacteria.

AMR surveillance should therefore have a prominent place in the strategic action plans of Member States to combat AMR. In 2011, about half of the countries within the European Region had well established national and international surveillance systems (mainly the countries of the European Union, affiliated with

EARS-Net), whereas these systems were often less developed in countries in eastern Europe and central Asia. To support countries outside the European Union in setting up or strengthening national AMR surveillance, the WHO Regional Office for Europe, the Netherlands National Institute for Public Health and the Environment (RIVM) and the European Society of Clinical Microbiology and Infectious Diseases (ESCMID), established the Central Asian and European Surveillance of Antimicrobial Resistance (CAESAR) network in 2012.

To further harmonize AMR surveillance methodology globally and enable standardized global reporting of official national AMR data, WHO launched in 2015 the Global AMR Surveillance System (GLASS). GLASS relies upon countries to conduct their own national surveillance and builds on the experience gained by long-standing regional AMR surveillance programmes, such as CAESAR and EARS-Net *(6)*. Through enhanced global surveillance and research of AMR, GLASS aims to inform decision-making by providing the evidence base for action and advocacy.

1.2 CAESAR network

As a network of national AMR surveillance networks, CAESAR supports establishment of national surveillance systems in eligible countries, i.e. all countries of the WHO European Region that are not members of EARS-Net. This aim – supporting establishment and strengthening of national AMR surveillance systems – is accomplished by improving national coordination, building laboratory capacity, and improving data quality. National AMR surveillance systems can evolve to include additional pathogens that are significant at local or national level and can continue to include more hospitals and laboratories.

The main objectives of the CAESAR network are to:

- encourage the implementation, maintenance, and improvement of national AMR surveillance programmes;

- support national systems in their efforts to improve diagnostic accuracy and quality at every level of the surveillance chain;

- collect comparable and validated AMR data to:

  - analyse trends over time

  - provide timely AMR data that constitute a basis for policy decisions

  - link to factors influencing the emergence and spread of AMR, such as antibiotic use data

- link the European scientific and professional community to exchange experience and expertise; and

- initiate, foster, and complement scientific research in Europe in the field of AMR.

Each of the countries within the CAESAR network is represented by its AMR focal point. This person is responsible for communication with the network coordinator, the coordination of activities and data reporting. Having an AMR focal point appointed, therefore, is a prerequisite for participation in CAESAR. By bringing together experts from different countries, each with their own expertise and views, the network facilitates exchange of experience and knowledge between professionals from a variety of countries and settings.

Please refer to section 3.1 for an overview of the roles and responsibilities of AMR focal points. A list of all AMR focal points is published yearly in the CAESAR annual report *(7)*.

1.3 CAESAR network coordination

CAESAR is a joint initiative of the WHO Regional Office for Europe, ESCMID, and RIVM (Fig. 1). The WHO Regional Office for Europe is responsible for overall programme management. RIVM provides technical assistance, methodology and data management of AMR surveillance and epidemiological expertise for data analyses. ESCMID provides microbiological guidance to ensure data quality and to improve laboratory standards, support in the implementation of appropriate quality assurance systems, and microbiological expertise in the analysis of data.

Fig. 1. CAESAR network coordination

The CAESAR network coordination group consists of two representatives from the WHO Regional Office for Europe, two from the ESCMID Study Group for Antimicrobial Resistance Surveillance (ESGARS-ESCMID) and two from the WHO Collaborating Centre for Antimicrobial Resistance Epidemiology and Surveillance of RIVM (RIVM):

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1.4 Surveillance stages

CAESAR supports setting up national AMR surveillance networks and improving laboratory quality, data management, analysis, and reporting in existing surveillance networks. Country support is tailored to the stage of maturity and specific needs of the respective surveillance system.

Stage 1. No national AMR surveillance system is in place and microbiological methods for the diagnosis of bloodstream infection are underutilized. A national AMR reference laboratory (NRL) may or may not be in place yet. Underutilization of microbiological diagnostics in routine patient care has been identified as an important obstacle for performing national AMR surveillance. In countries in stage 1, the focus is on building laboratory capacity and diagnostic stewardship through Proof-of-Principle routine diagnostic projects for AMR surveillance (PoP projects) *(8,9)*. During a PoP project, three to five hospitals in a country will systematically assess the antimicrobial susceptibility patterns in the most important pathogens causing community-acquired and hospital-acquired bloodstream infections. The main goal of the project is to contribute to improving clinical care for patients admitted with suspected bloodstream infections, in accordance with evidence-based medicine, thereby making use of microbiological diagnostics. Furthermore,

the project aims to: demonstrate to clinicians the value of clinical microbiology as part of the diagnostic work-up of patients with suspected bloodstream infections; establish and support a surveillance network as a starting point for a functional national laboratory-based surveillance system for AMR; and provide insight into antibiotic resistance levels in a country. Throughout the duration of the project, support is provided by a team of consultants and through a protocol including standard operating procedures.

Stage 2. Antibiotic susceptibility testing is already routinely performed in clinical settings, but data are not yet collected and analysed systematically at national level. In such countries the emphasis is on setting up a surveillance network and promoting standardized data collection in parallel with harmonization of laboratory methods. Another point of emphasis is building capacity of an NRL to provide reference function and quality control for the network.

Stage 3. Countries in stage 3 have an established and functional surveillance system in place with a national reference laboratory fulfilling typical reference functions. In these countries, the focus is on harmonizing laboratory methods, expanding representative coverage, and streamlining data management.

1.5 Support provided by CAESAR

The WHO Regional Office for Europe and partners support countries with the stepwise approach towards national surveillance and coordination of AMR activities. Continued support and collaboration within the CAESAR network among countries and partners is fundamental in the process of building a network of national AMR surveillance systems in all countries of the European Region.
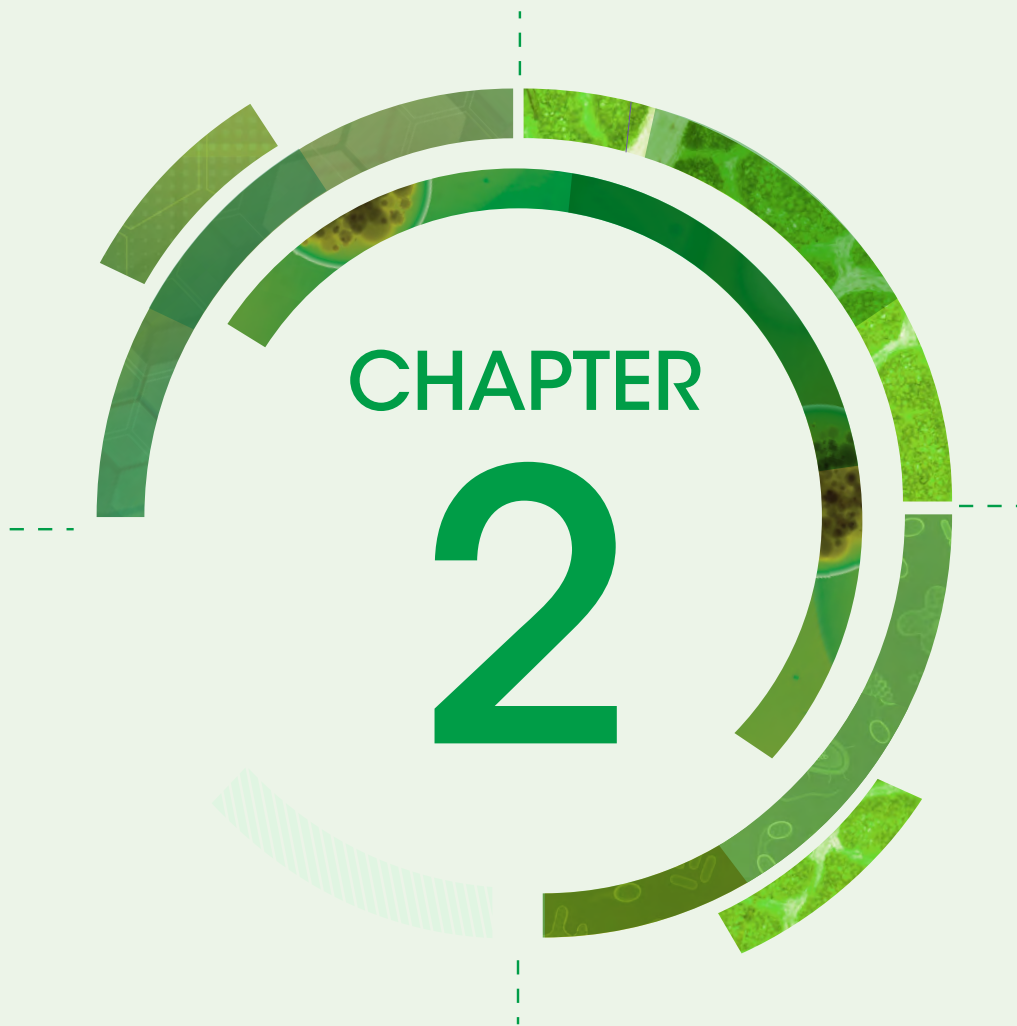
Support can be provided in the form of multi country and national workshops and consultancies to set up or to strengthen national AMR surveillance systems, focusing on various aspects:

- national coordination, stakeholder meetings, and development of national AMR action plans;

- principles of laboratory quality control, the use of standard operating procedures and European Committee on Antimicrobial Susceptibility Testing (EUCAST) *(10)* guidelines, and interpretation of antimicrobial susceptibility testing (AST) data;

- the tasks of an AMR reference laboratory in terms of national coordination of the laboratory network, quality assurance, and confirmation of test results; and

- surveillance methodology, principles of data collection and data management according to CAESAR methodology, data analysis, and interpretation of AMR surveillance data.

The support efforts of the CAESAR network include:

- publication of the CAESAR manual and technical manuals providing guidance on data collection and management and on the interpretation of AST data, available on the Regional Office website *(7)*;

- support to laboratory networks making the transition to EUCAST methodology;

- training courses on laboratory quality management;

- support in setting up laboratory quality assurance systems;

- training for AMR reference laboratories;

- annual external quality assessment (EQA) exercises;

- support in setting up and improving standardized (electronic) data collection, data management, and data quality assurance procedures;

- technical and financial support for the organization of the national AMR surveillance network in order to foster discussion about local surveillance data and EQA results, as well as efforts to improve surveillance and to assess capacity building needs;

- annual CAESAR network meetings, for which all AMR focal points from CAESAR participating countries are invited to discuss AMR trends, network progress, EQA results, and specific issues and challenges related to AMR surveillance; and

- publication of the CAESAR annual reports, available on the Regional Office website *(7)*.

CHAPTER

2

CAESAR methodology

This chapter describes the CAESAR approach to surveillance and the CAESAR methodology. It presents the factors that may affect the quality of the data, as well as levels of evidence to assess the quality and representativeness of data. Lastly, expanding surveillance beyond CAESAR is briefly discussed.

2.1 The CAESAR approach to surveillance

Several approaches to AMR surveillance exist, each with its specific objectives, strengths, and weaknesses *(11)*. CAESAR aims to collect reliable population-level data on AMR within a country or area, which may constitute a basis for policy decisions. The CAESAR methodology is a passive, laboratory-based surveillance system used to collect routine AST data of invasive isolates. Using routine AST data has several advantages. Assuming that blood and cerebrospinal fluid (CSF) cultures are taken routinely in clinics in case of suspected bloodstream infection or meningitis, no changes in the diagnostic processes are needed. Other advantages of using routine AST data are the continuous availability of data, the cost saved by double use of the data – once for clinical practice, once for surveillance – and the opportunity to assess laboratory quality. Furthermore, when using aseptic sample collection methods, any bacterial isolate of the species under surveillance found in a sample taken from a normally sterile body fluid may be considered a pathogen. Including routine non-invasive isolates may produce incomparable results for surveillance purposes as processing of such samples is heavily influenced by clinical interpretation that varies from country to country.

CAESAR is collaborating closely with EARS-Net, hosted by ECDC, and has adopted the same standards and methodologies. This approach allows for joint presentation and comparison of AMR data for all countries of the WHO European Region.

2.2 Pathogens under CAESAR surveillance

Nine (groups of) pathogens are selected for surveillance of AMR:

- *Escherichia coli (E. coli)*

- *Klebsiella pneumoniae (K. pneumoniae)*

- *Salmonella* species (spp.)

- *Pseudomonas aeruginosa (P. aeruginosa)*

- *Acinetobacter* spp.

- *Staphylococcus aureus (S. aureus)*

- *Streptococcus pneumoniae (S. pneumoniae)*

- *Enterococcus faecalis (E. faecalis)*

- *Enterococcus faecium (E. faecium).*

E. coli, K. pneumoniae, P. aeruginosa, Acinetobacter spp., *S. aureus, S. pneumoniae, E. faecalis,* and *E. faecium* are pathogens under CAESAR surveillance according to the EARS-Net reporting protocol. These pathogens are selected because they are common causes of invasive infections, and when they develop AMR, therapeutic options may be severely limited. In addition, these pathogens have the potential to spread in the community or within and between health care settings. These pathogens are therefore of public health and clinical importance.

In order to further align CAESAR methodology with that of GLASS, CAESAR added *Salmonella* spp. as a pathogen under surveillance in 2016 *(12)*.

For a more detailed description of the pathogens under CAESAR surveillance, please refer to Annex F. For a list of the antimicrobial groups under surveillance for each pathogen, please refer to Chapter 6.

2.3 Selection of surveillance sites and source population

An AMR surveillance system following CAESAR methodology aims to provide the average susceptibility pattern of bacteria in patients presenting with a bloodstream or central nervous system infection in the entire country/area (the target population).

When starting national AMR surveillance, collecting data from the entire country, including all health care facilities and laboratories, is often not feasible. In a sentinel surveillance system, data are only collected from a subset of health care facilities and laboratories (the AMR surveillance sites). In order to obtain a nationally representative assessment of AMR, the AMR surveillance sites selected for participation in the national surveillance should be from different geographical and climatic regions, including both rural and urban areas. In addition, health care facilities should reflect a mix of hospital types (e.g. tertiary, general, and paediatric hospitals) within the country/area.

In addition, the sample of patients included in surveillance ideally consists of a mix of patient types and infection types, in proportion to their occurrence in the total population. Selective inclusion of patients from certain hospital departments (e.g. intensive care units (ICUs)) or institution types (e.g. tertiary care institutions), patients with certain infection types (e.g. community-acquired urosepsis or health care-associated bloodstream infections), patients with chronic or recurring infections, or patients with relapses or treatment failure will likely overestimate the resistance proportion because these patients may have been subjected to selective pressure of antimicrobials.

It is important to keep in mind that data used in passive surveillance, as within the CAESAR network, are not generated with surveillance as the primary objective, but with patient care as the primary aim. Clinical predictions are included in the decision on whether to take a sample for microbiological diagnostics. As a consequence, more severely ill patients, patients among whom treatment is problematic, or patients suspected of having antibiotic-resistant infections will likely always be overrepresented. Sampling from these specific patient categories will allow the generalization of results to that specific population, but not necessarily to the overall patient population.

If possible, samples should always be taken prior to initiation of antimicrobial therapy. Sampling after antimicrobial therapy has already been started or following self-treatment in settings where over-the-counter sales of antibiotics is common results in an underrepresentation of infections that respond to first-line antibiotics.

2.4 Processing of samples in the laboratory, data collection, and data analysis

Producing high-quality AST data requires a sufficient quality of culturing and AST materials in the laboratories, the use of standard operating procedures and guidelines, and laboratory personnel adequately trained to use these materials and documentation.

To ensure accurate results, AST must be done according to well developed and scientifically grounded standards. The use of international guidelines for susceptibility testing including harmonized breakpoints is a means to ensure comparability between AST in different laboratories and countries. Both EUCAST and the Clinical and Laboratory Standards Institute (CLSI) provide comprehensive methodological standards for routine AST, confirmatory testing and their interpretation. CAESAR recommends countries to use either EUCAST or CLSI standards. Since EUCAST guidelines are the most widely used in the WHO European Region and all EUCAST documents are freely downloadable in various languages *(10)*, CAESAR provides training in EUCAST methodology. Laboratory methods including AST methodology are described in detail in Annex B.

A laboratory quality management system and regular application of internal quality assurance procedures allow the timely detection and correction of errors in laboratory procedures. National auditing and accreditation schemes in conjunction with external quality assurance programmes ensure that laboratories conform to national quality standards. The CAESAR network, jointly with EARS-Net, organizes annual EQA exercises in which all CAESAR laboratories are invited to participate.

CAESAR recommends collecting AST data throughout the year, thereby avoiding the influence of outbreaks of antibiotic-resistant bacteria or seasonal variation on the observed resistance proportions. A uniform test panel for AST including indicators of susceptibility to all antimicrobial groups should be tested for all bacterial isolates, as listed on the minimum reporting panel (Chapter 6). Data should be collected systematically, using standardized and uniform codes. Several data collection tools are available through the CAESAR network. Collected data should be checked for completeness and quality on a regular basis. Data collection methods are discussed in detail in Chapter 4.

Individual patients are often sampled repeatedly during their illness, for diagnostic purpose or to assess therapeutic response. Patients with infections caused by resistant microorganisms are more likely to be sampled more than once. Inclusion of repeat isolates from an individual patient in data analysis will result in overestimation of the resistance proportion, because the resistant isolates are overrepresented. To prevent this, CAESAR includes only the first isolate per microorganism per person per year in analyses – the convention when doing surveillance *(13)*. If on one and the same day a certain pathogen is isolated from both blood and CSF from a single patient, only data on the CSF isolate are included in analyses.

RIVM analyses CAESAR data and presents the results as the percentage of tested isolates "resistant" (R) and "susceptible, increased exposure" (I) to an individual antibiotic or an antibiotic group. Details on data analysis are discussed in Chapter 6.

2.5 Epidemiological data

In addition to isolate-level data, including results from species identification and susceptibility testing, demographic data about the patient are collected within CAESAR. These data allow AMR figures to be linked to specific patient groups. Details on population denominators (an estimate of the catchment population of the hospital) and characteristics of participating laboratories and hospitals (e.g. the number of blood culture requests, the number of beds, and the level of care) are collected as well. Together they facilitate interpretation of isolate-level data and help to assess the accuracy and representativeness of these data.

Data collection methods are discussed in detail in Chapter 4. An overview of all variables collected within CAESAR is in Annex E.

2.6 Data quality

Data quality may be negatively affected during each of the phases in the data generation process discussed in the previous sections: the selection of surveillance sites that participate in the surveillance programme, the selection of patients for blood culturing in the hospital, the processing of samples in the laboratory, and the aggregation and analysis of the data.

Deviations from the truth may be due to either random or systematic errors. Both of these types of errors affect data accuracy. Random deviation results from chance variation occurring during sampling or measurement. Systematic deviation is caused by systematic errors in collecting, processing, and analysing the data. Systematic deviation is also called bias. In particular, systematic deviation may occur because of choices made when taking patient samples (sampling bias), when processing samples in the laboratory (measurement error), or when aggregating data for analysis (such as including multiple isolates from the same species in the same patient in the same calendar year). Random error will always occur and can be reduced to a certain extent. In contrast, systematic error can be significantly reduced by closely following and improving the data generation process.

The several sources of error and bias in AMR surveillance data, as well as possible strategies to prevent these, are summarized in Table 1.

Table 1. Sources of error and bias in AMR surveillance data

Type of error/bias		Mechanism	Solution
Random error	Sampling variation	Chance	Increase sample size
	Measurement variation	Test-to-test variation in application of laboratory procedures	Increase sample size Standardize procedures Provide continuous training of laboratory staff Set up quality assurance systems
Systematic error	**Bias due to sampling procedures**		
	Selection of participating sites	Sampling specific patient populations only, such as tertiary hospitals, ICUs, and urban centres	Select a mixture of hospital types and departments from different geographical regions
	Selection of patients	Sampling only severe cases or after treatment failure	Improve case ascertainment; promote sampling of all cases with signs of bloodstream infection prior to treatment initiation (active case finding)
	Bias due to laboratory procedures		
	Laboratory standards	Use of non-uniform AST methods, such as breakpoints from product inserts and out-of-date standards Sequential testing, such as testing susceptibility for carbapenems only if isolate is resistant to third-generation cephalosporins	Use national standards based on international standards for AST methodology (such as EUCAST) Test susceptibility to all indicator antimicrobials (uniform test panel) on all microorganisms
	Measurement error	Improper application of laboratory methods, such as use of non-standard inoculum Inadequate laboratory materials, such as use of expired or non-quality controlled antimicrobial disks Damaged and/or poorly calibrated equipment, such as out-of-date firmware used with automated systems	Provide continuous training of laboratory staff Set up laboratory quality assurance systems Perform confirmatory testing of multidrug-resistant microorganisms or exceptional antimicrobial resistant phenotypes Procure high-quality and quality-controlled materials
	Bias from data aggregation and analysis procedures		
		Include repeat isolates from individual patients Use of varying expert rules, such as different rules for deriving resistance used in each laboratory	Collect non-aggregated (raw) data Use standardized data aggregation and analysis methods

2.7 Levels of evidence

Based on the quality and representativeness of the collected AMR surveillance data, a qualitative assessment can be made about the level of evidence of these data. For the CAESAR report, a yearly assessment for each country or area is made, to guide interpretation of the data presented in the report. Factors related to the surveillance system, sampling procedures, and laboratory procedures are taken into account to arrive at the level of evidence.

1. **Surveillance system**
   a. geographical coverage (Are all major geographical regions represented?)
   b. selection of surveillance sites (Are all major hospital types represented?)

2. **Sampling procedures**
   a. selection of patients (Are all major patient groups presenting with suspected invasive infections sampled?)
   b. sample size (Are at least 30 isolates per pathogen available?)

3. **Laboratory procedures**
   a. AST methods (Are all isolates tested for each relevant antibiotic group and using current methodological standards? Is a national quality assurance system active?)
   b. AST breakpoints (Is a harmonized and up-to-date breakpoint system used?)

Three levels of evidence have been defined.

Level A data provide an adequate assessment of the magnitude and trends of AMR in the country/area.

Level A data allow for the valid and reproducible assessment of AMR trends in the country/area. The data can be used to raise awareness about AMR and as input for developing AMR control policies. However, the resistance proportions as included in the CAESAR report should not be used as the sole source for informing empirical treatment choices, as the total sample of patients comprises a mix of community-acquired and health care-associated infections in different types of patients. To guide empirical treatment, more comprehensive and clinically well characterized local AMR surveillance data are needed, to allow the assessment of resistance patterns in specific patient populations (such as children or ICU patients), specific infection types (such as community-acquired versus health care-associated, urosepsis versus central line-associated bloodstream infection versus severe pneumonia) and treatment status (before and after empirical antibiotic treatment).

Level B data provide an indication of resistance patterns present in clinical settings in the country/area, but the proportion resistance should be interpreted with care. Improvements are needed to attain a more valid assessment of the magnitude and trends of AMR in the country/area.

Level B data are not necessarily wrong but rather less generalizable to the target population due to systematic errors or biases in the data generation process. Presenting and publishing level B surveillance data is important, as it enables critical appraisal of the sources of error and bias, and is a point of departure for further improvements. The magnitude of resistance presented is biased and thus precludes the data from being used for guiding empirical antibiotic treatment choices. However, the data do indicate the presence of multidrug-resistant microorganisms or exceptional antimicrobial resistant phenotypes of public health importance (e.g. carbapenem-resistant *Enterobacterales*) in clinical settings in the country/area. Although further research is needed to assess the extent of the problem and the spread of these microorganisms in the health care system, the data indicate that infection prevention and control measures are acutely needed to control the problem.

Level C data do not provide an adequate assessment of the magnitude and trends of AMR in the country/area. The current basis for data collection requires targeted improvements to allow a valid assessment of the AMR situation.

Level C data should not be used to inform empirical antibiotic treatment choices or AMR control policy. The data do not provide an adequate assessment of the AMR situation in the country/area due to substantial errors in AST. However, the surveillance system has shown the capacity to collect routine AST data from a network of laboratories. The current basis for data collection requires targeted improvements to allow a valid assessment of the AMR situation.

2.8 Expanding surveillance beyond CAESAR requirements

CAESAR provides a starting point for national AMR surveillance, only collecting data on invasive isolates for a limited number of pathogens. Once surveillance based on invasive isolates has become routine and the quality of data is assured, countries are encouraged to gradually expand the number of participating sites in the network to improve geographic coverage. Surveillance sites included in the primary surveillance network can support the engagement and development of new sites. Furthermore, the network can incorporate in its AMR surveillance system additional pathogens, antimicrobials, and other types of clinical specimen cultures (e.g. urine, pus) that are of local or national importance.

Countries submitting data to CAESAR are also encouraged to start contributing data to GLASS. GLASS collects AMR data, aggregated at national level, thereby building on national and regional networks. The goal of GLASS is to collect and analyse standardized, comparable and validated AMR data, in order to inform decision-making; drive local, national and regional actions; and provide the evidence base for action and advocacy. GLASS has a wider scope in terms of species and specimen types than CAESAR. In addition to collecting data on the CAESAR pathogens in blood samples, GLASS also collects data on *E. coli* and *K. pneumoniae* in urine, *Shigella* spp. and *Salmonella* spp. in stool, and *Neisseria gonorrhoeae* in urethral and cervical swabs *(14)*.
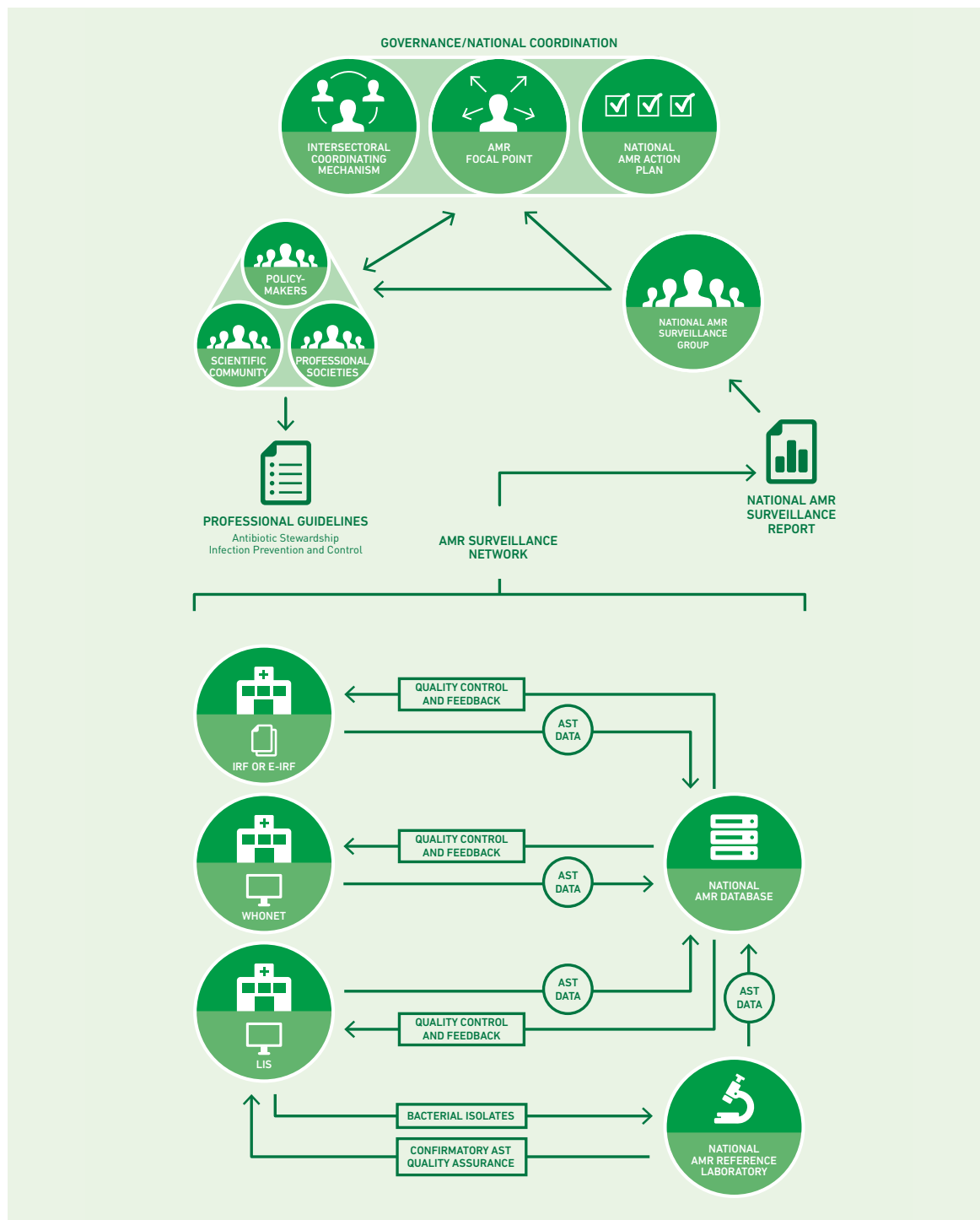
On behalf of the countries enrolled in GLASS and with their approval, the CAESAR network provides aggregated AMR country data to GLASS to avoid double reporting and an additional burden on network members. Members of the CAESAR network are encouraged to report AMR data on pathogens and specimens not included in CAESAR directly to GLASS.

CHAPTER

3

Organization of a national AMR surveillance system

Many different professional levels are involved in a national AMR surveillance system. This chapter introduces the various institutions and professionals involved in the system, and their roles and responsibilities. Fig. 2 provides an overview of the institutions that should be involved in the system.

Fig. 2. National AMR surveillance system

GOVERNANCE/NATIONAL COORDINATION

INTERSECTORAL COORDINATING MECHANISM

AMR FOCAL POINT

NATIONAL AMR ACTION PLAN

POLICY-MAKERS

SCIENTIFIC COMMUNITY

PROFESSIONAL SOCIETIES

NATIONAL AMR SURVEILLANCE GROUP

PROFESSIONAL GUIDELINES
Antibiotic Stewardship
Infection Prevention and Control

AMR SURVEILLANCE NETWORK

NATIONAL AMR SURVEILLANCE REPORT

IRF OR E-IRF

QUALITY CONTROL AND FEEDBACK

AST DATA

WHONET

QUALITY CONTROL AND FEEDBACK

AST DATA

NATIONAL AMR DATABASE

LIS

AST DATA

QUALITY CONTROL AND FEEDBACK

AST DATA

BACTERIAL ISOLATES

CONFIRMATORY AST QUALITY ASSURANCE

NATIONAL AMR REFERENCE LABORATORY

(e-)IRF: (electronic) isolate record form; LIS: laboratory information system.

3.1 Governance and national coordination

The resolutions accompanying the European strategic action plan on antibiotic resistance and the global action plan on antimicrobial resistance urge Member States to ensure political commitment and resources for implementing national strategic action plans on AMR. The global action plan furthermore states that AMR surveillance activities should be incorporated in the action plans. The WHO Regional Office for Europe and partners are working together with Member State governments to develop and implement these comprehensive strategic action plans.

To set up a continuous and sustainable national AMR surveillance system, government support is needed on legal, technical, and financial aspects. Implementing these activities will require long-term investment, for instance in operational research, laboratories, human and animal health systems, competent regulatory capacities, and professional education and training, in both the human and animal health sectors *(3)*. The ministry of health is one of the main entry points, responsible for providing a mandate to a national institute to develop a national AMR surveillance system. A public health institute usually undertakes this function; other institutes (e.g. research institutes) may be considered more suitable, but they must have access to both laboratory and epidemiological expertise and have a defined structure for surveillance coordination and data management. In addition, the ministry of health is responsible for providing a legal basis for centralized collection of patient data at national level.

The ministry of health appoints a national AMR focal point, responsible for the coordination of the national surveillance system on AMR (Fig. 3). The responsibilities of the AMR focal point include setting up and/or sustaining an intersectoral coordination mechanism for AMR (e.g. taskforce, steering committee, board, or council). The intersectoral coordinating mechanism should encompass representatives of the relevant stakeholders for AMR (e.g. ministry of health, ministry of agriculture, national AMR reference laboratories, public health institutes, national medicines agency, academia, nongovernmental organizations, and the private sector). Through the intersectoral coordinating mechanism, the national AMR focal point facilitates effective communication, information sharing and coordination between relevant stakeholders for AMR. Another responsibility of the AMR focal point is to monitor the development of a national AMR action plan (including objectives, policies, surveillance, and control). Having an AMR focal point appointed is a prerequisite for participation in the CAESAR network.

Fig. 3. Governance/national coordination

INTERSECTORAL COORDINATING MECHANISM

AMR FOCAL POINT

NATIONAL AMR ACTION PLAN
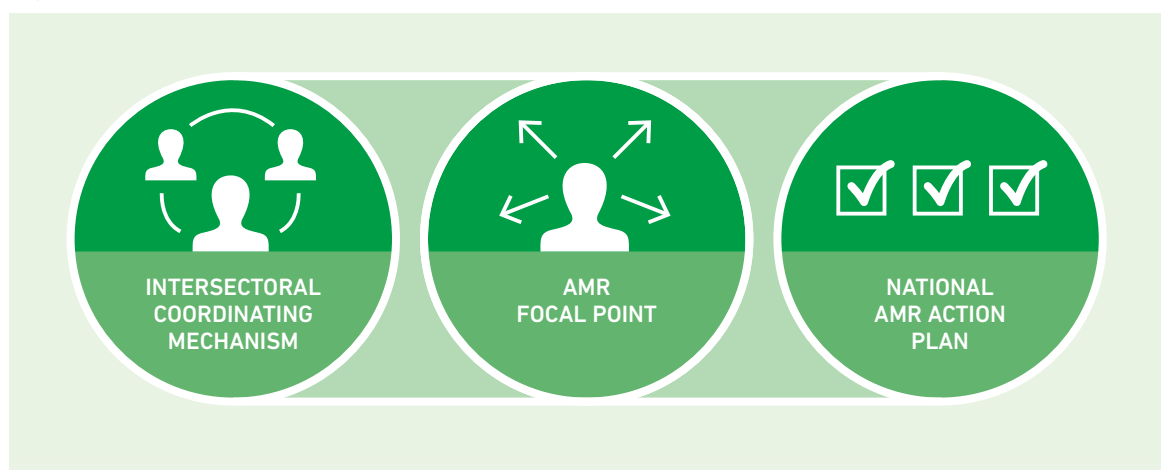
3.2 National AMR surveillance group

The AMR focal point coordinates and supports the national AMR surveillance group. The national AMR surveillance group is typically based at a national public health or a research institute – referred to as

the national coordinating centre in the GLASS manual *(14)*. It is possible to issue a letter of agreement between the WHO Regional Office for Europe, on behalf of the CAESAR network, and the institute where the AMR surveillance group is based. The letter of agreement defines the terms of the collaboration between the CAESAR network and the national entity. The national AMR surveillance group is responsible for overseeing and coordinating the national AMR surveillance system. Ideally, in addition to the AMR focal point, this group also consists of the following specialists: an epidemiologist (with expertise in data analysis and interpretation), medical microbiologist, infectious disease specialist, and a data manager. The medical microbiologist may be based at or have ties to the NRL, ensuring close collaboration between the national AMR surveillance group and the NRL. Fig. 4 summarizes the group's responsibilities, which include the following:

- Develop and disseminate national protocols for data collection and data management, laboratory management, and diagnostic stewardship. This CAESAR manual can be used as a basis for developing or adapting national protocols.

- Coordinate and support data collection. This includes providing technical support and training to surveillance sites and, in close collaboration with the NRL, making sure quality control is conducted within the laboratories (internal quality assurance).

- Monitor and evaluate the national AMR surveillance system. This includes checking regularly data completeness and data quality, and providing feedback to hospitals and laboratories within the network.

- Maintain the national AMR database.

- Analyse national surveillance data.

- Disseminate the results of the analysis to relevant stakeholders, including the ministry of health and directors of participating hospitals, by producing yearly national surveillance reports and organizing yearly AMR surveillance meetings. During meetings, all involved stakeholders discuss the surveillance results, thereby improving the network's awareness of the national situation, leading to better use of surveillance data in clinical care, for the development of guidelines, and for decision-making at political level. Discussion of the results also provides an opportunity to improve data collection.

- Collaborate and participate in international networks, such as CAESAR and GLASS. This includes preparing and submitting a national dataset to the CAESAR network. It also means providing the CAESAR network with an estimate of the population coverage of the AMR surveillance sites participating in the national AMR network and generating data for CAESAR and of laboratory and hospital characteristics, using standardized CAESAR questionnaires (Annex A).

The AMR surveillance group should encourage the participation of laboratories and hospitals to ensure a good representation of the population. After gaining experience with AMR surveillance, based on routine diagnostics from a selection of the country's hospitals and laboratories establishing the basis for sustained AMR surveillance, the national AMR surveillance group should aim to expand its network to improve geographic coverage (see also section 2.3). In order to recruit new AMR surveillance sites, key staff members from eligible laboratories expressing an interest in national surveillance may be invited to a meeting in order to understand the aims of national AMR surveillance, the network, and the requirements of participating in it. The CAESAR network can provide support in the form of PowerPoint presentations or the presence of a member of the CAESAR network pool of consultants in such a meeting. Prior to the start of data collection, laboratory personnel should be trained on the principles of data collection and data management according to CAESAR methodology, as well as the objectives of surveillance. The CAESAR network coordination group and an international pool of consultants are available to help develop and provide this training.

Fig. 4. Roles and responsibilities of the national AMR surveillance group

3.3 National AMR reference laboratory

Every participating country is encouraged to appoint an NRL to fulfil the responsibilities listed below. The national AMR surveillance group explores which public health or academic laboratories have the expertise in characterization and confirmatory testing of antimicrobial resistant pathogens and are interested in taking on the role of the NRL.

Trainings, supported by CAESAR, can be given in countries where no laboratory reaches the level of expertise necessary for taking on the role of NRL. If no laboratory within the country qualifies for the function of NRL, regional collaboration can be established with an appropriate institute in a neighbouring country.

Fig. 5 summarizes the responsibilities of the NRL, which include the following:

- Develop methodological standards to be used in AMR surveillance sites.

- Develop a national laboratory quality assurance system.

- Help organize the participation of AMR surveillance sites in external quality assurance schemes including the yearly CAESAR EQA exercise.

- Perform additional AST, which may include confirmatory testing for unusual AMR results, testing of resistance mechanisms, and molecular testing.

- Provide guidance and technical support in AST and quality assurance, and support for capacity building. The NRL may, for example, provide training in AST and quality management for laboratories within the surveillance networks and new laboratories.

Fig. 5. Roles and responsibilities of the AMR reference laboratory

3.4 AMR surveillance sites

The national AMR surveillance system consists of a network of clinical laboratories (AMR surveillance sites) periodically submitting AST data to the national data manager. To be eligible for participation in the national AMR surveillance system, a laboratory needs to be able to process and share the results of routinely collected clinical samples. The laboratory may be directly associated (on site) with a hospital at which blood and CSF samples are collected as part of standard care for patients with infections. Alternatively, a laboratory may serve several health care facilities, thereby performing the role of a central testing facility. In the latter case, samples should be correctly stored at the hospital and transport should be well organized.

Species identification and AST are performed at the AMR surveillance sites. Please refer to Annex B for a detailed description of AST methodology.

Subsequently, AST results and data at patient level should be collected in a standardized format. Data should be shared with the national AMR surveillance group allowing the national data manager to check the data quality on at least a quarterly basis. Details on data collection and submission to the national AMR surveillance group are discussed in Chapter 4. Details on the pathogen–antibiotic combinations on which data should be collected are in Chapter 6.

CHAPTER

4

Data collection

This chapter presents methods for data collection, data management, and data sharing within the national AMR surveillance system.

## 4.1 AMR surveillance sites

4.1.1 Data at isolate level

Species identification and AST should be performed at each of the AMR surveillance sites, and results should be recorded at the site itself. Several tools can be used to record isolate level data at the AMR surveillance site and subsequently submit data to the national AMR surveillance group (Fig. 6). The CAESAR network provides paper and electronic IRFs (eIRFs), which can be adapted for local or national use. Other options are the use of WHO microbiology laboratory database software (WHONET) or a laboratory information system (LIS). Which method is best depends on several factors and can be decided in consultation with the CAESAR data manager (caesar@rivm.nl).

4.1.1.1 Isolate record forms
IRFs provide a convenient method to collect AST results for surveillance. Annex C provides paper IRF templates, containing the minimum panel of antibiotics selected for CAESAR reporting. These forms serve as a template and can be adapted to fit the national surveillance system guidelines and local procedures.

Fig. 6. An overview of sharing samples and data in the national AMR surveillance network
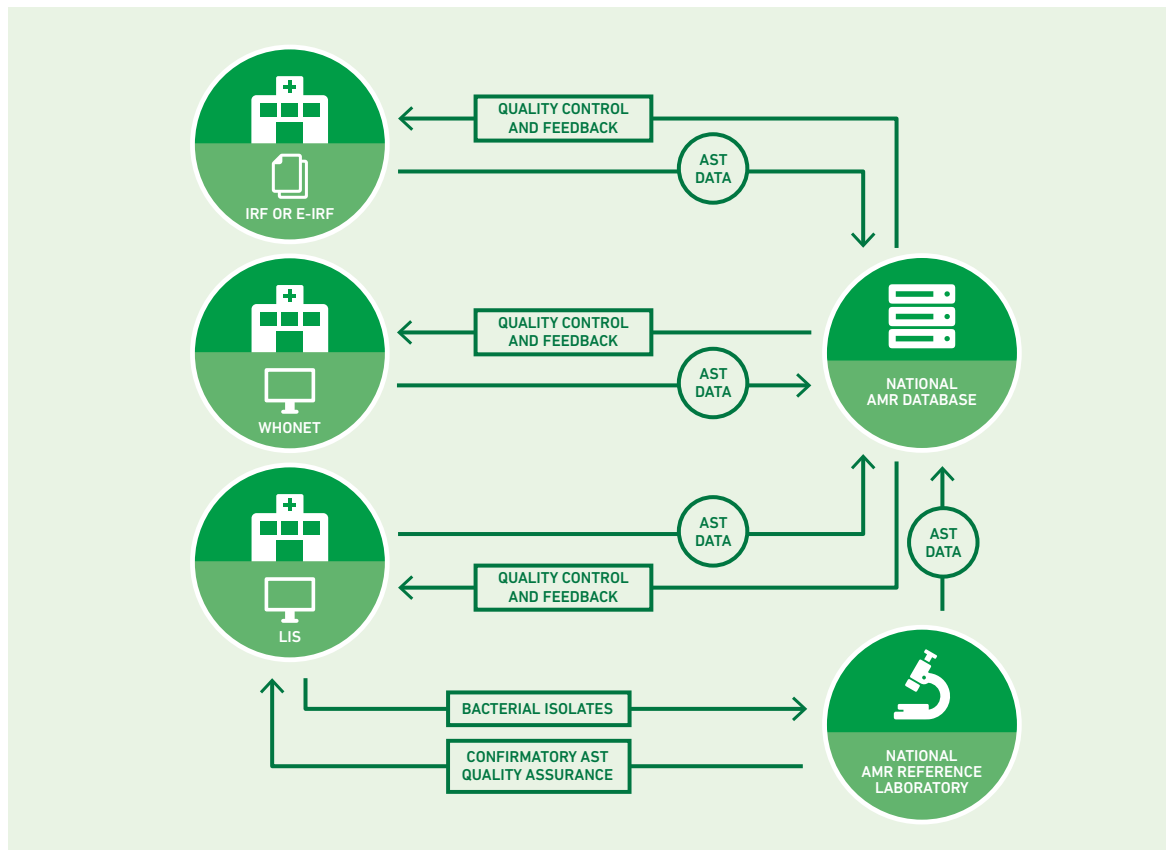
(e-)IRF: (electronic) isolate record form; LIS: laboratory information system.

The CAESAR minimum panel of antibiotics in some cases contains several equivalent antibiotics (e.g. meropenem or imipenem); countries can choose to include one of these antibiotics in their national AST guidelines. The redundant antibiotics, those that will not be tested in the laboratory, can be removed from the IRFs. Likewise, IRFs can be extended with additional antibiotics that are important for local or national surveillance.

The IRFs are also available electronically as a Microsoft Excel file, enabling electronic capture of data at the AMR surveillance sites without the need for a dedicated LIS. These eIRFs have the same structure as the paper forms, and can similarly be adapted to the local situation. The data form can be configured to any language, and any user can perform the required translations, without the need for programming skills. Data are stored in the Microsoft Excel (.xlsx) spreadsheet format. More information on using and configuring eIRFs is in Annex D. The eIRF is available from the CAESAR data manager by email (caesar@ rivm.nl).

4.1.1.2 WHONET
WHONET is a free Windows-based database program that is downloadable from the WHONET website *(15)*. CAESAR recommends laboratories to use WHONET as a starting point for routine electronic collection of AST data in hospital laboratories. This software was developed to manage and analyse microbiology laboratory data, focusing on AST data at institution level. WHONET also includes an automated function to export datasets in the CAESAR data format. Practical guidance on how to use WHONET for CAESAR is in the manual WHONET for CAESAR *(16)*.

4.1.1.3 Laboratory information system (LIS)
Another option for recording laboratory results is using a LIS. Laboratories that record results using a LIS have several options to export data for AMR surveillance from it. Please refer to section 5.3 for a discussion of the options.

4.1.2 Data at the level of the laboratory and hospital

Having background information on the patient population and on sampling practices in hospitals is important when analysing AMR surveillance data. This information provides a better understanding of the resistance proportions within the local context and enables calculation of resistance incidence. This information also allows the assessment of the representativeness of the national AMR surveillance system as a whole.

When a country starts to submit data to CAESAR, the AMR focal point is asked to complete a small questionnaire for every participating laboratory and hospital, and then to provide yearly updates. Laboratory and hospital questionnaires are included as Annex A.

The laboratory questionnaire contains questions on the location of the laboratory and the CAESAR hospital codes for the hospitals served by the laboratory. This information is used to determine the geographic distribution of the laboratories and hospitals participating in the network. Furthermore, laboratories are asked to provide the total number of blood culture sets analysed in the past year for each hospital.

The hospital questionnaire contains questions on the level of care provided by the hospital (to get insight in the patient population), the number of beds, the number of ICU beds, the number of patient days per year, and the number of yearly admissions.

The number of blood culture sets analysed in relation to the total number of hospital beds or patient days gives an indication of the blood sampling rates within a hospital.

4.2 The national AMR reference laboratory

For the purpose of quality control or confirmatory testing of multidrug-resistant microorganisms or exceptional antimicrobial resistant phenotypes, bacterial isolates may be sent from each of the AMR surveillance sites to the NRL. Results are sent back to the AMR surveillance sites and may be used for quality assurance.
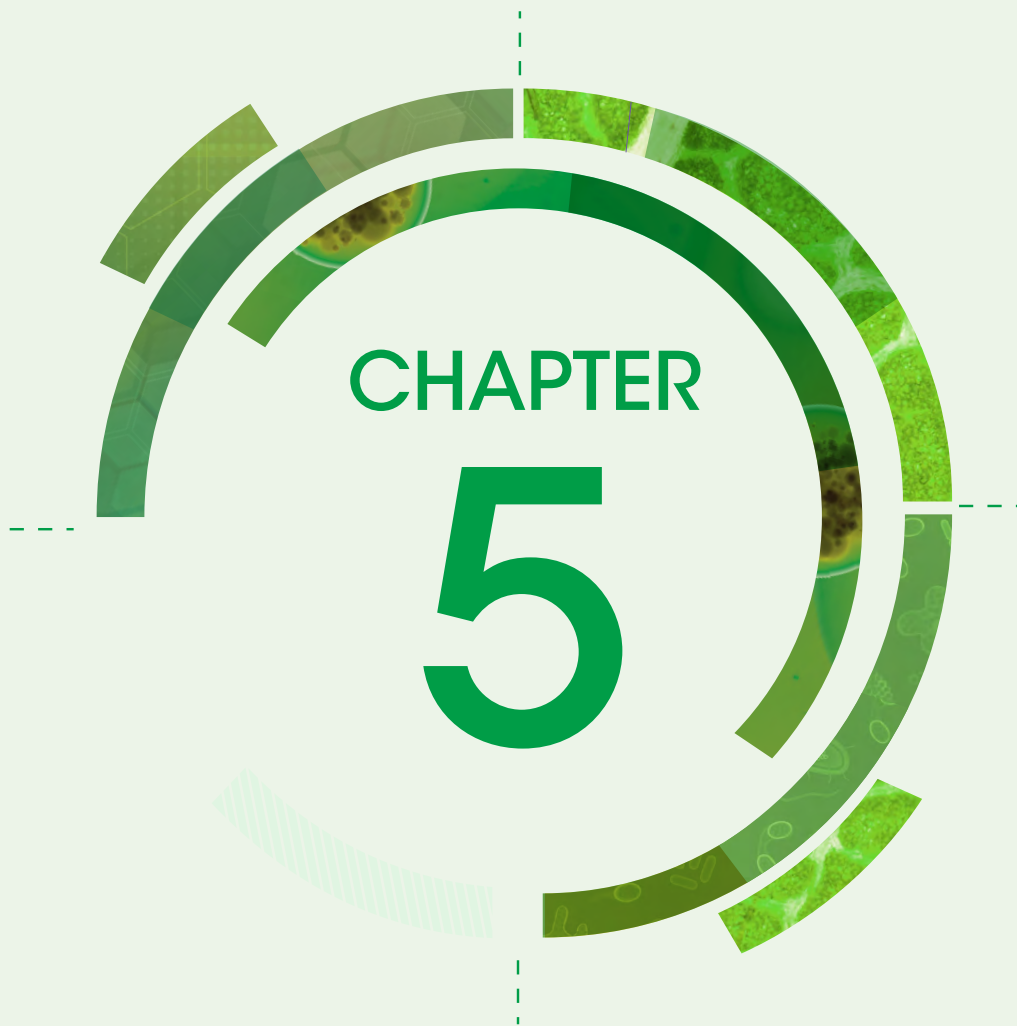
At the NRL, AST results are recorded similarly as in the AMR surveillance sites, either through paper or electronic IRFs, directly into WHONET, a LIS, or another database.

4.3 Feedback from the national AMR surveillance group

Timely feedback of problems with the data allows for improvement of the national surveillance system. AMR surveillance sites as well as the AMR reference laboratory periodically submit data to the national AMR surveillance group. Paper and electronic IRFs may be sent directly to the national data manager; data should be exported if WHONET or a LIS is used. Laboratories should submit all data specified for the pathogen–antibiotic combinations under surveillance (Chapter 6) to the national data manager. Prior to submission, laboratories should check data for:

- completeness of data, including patient information;

- adherence to local and national standards for bacterial culturing, species identification, and antibiotic susceptibility testing;

- microbiological consistency/plausibility; and

- consistency with clinical S/I/R breakpoints according to the guideline used (issued by an appropriate board or committee, e.g. EUCAST (10) or CLSI (17)).

The national data manager checks the submitted AMR data from AMR surveillance sites and the NRL, and then provides feedback on data completeness and quality, and, if necessary, asks for an updated file.

CHAPTER

5

Preparation and submission of national datasets to CAESAR

To participate in the CAESAR international surveillance system, data from the national surveillance network have to be submitted to the CAESAR data manager. Technical requirements for the dataset to be submitted (the CAESAR dataset) and the submission and feedback procedures are described in sections 5.1 and 5.2.

The information presented in sections 5.3 and 5.4 is especially relevant in countries that set up a national AMR surveillance system in the context of participating in the CAESAR network. These sections provide guidance on creating the CAESAR dataset and on data security and anonymization respectively.

5.1 Structural and technical requirements of a CAESAR dataset

Data on all strains isolated from blood cultures and CSF cultures in which AST has been performed may be included in the CAESAR dataset.

Annex E contains an overview of the variables to include in the CAESAR dataset. The variables may be in any order, but the first row (header) of the dataset file must contain the names of the variables. Furthermore, variable names must be spelled exactly as described in Annex E. The software that imports the data into the CAESAR database is not case-sensitive, so capitalization of these names is not important.

The required format for the CAESAR dataset is a "comma-separated values" (CSV) text format. Fields, including the headers, may be separated by a comma (","), a pipe ("|"), or a semicolon (";"). The same separator must be used consistently throughout the file. Each line in the CAESAR dataset should contain data on only one isolate–antibiotic combination, which means that results on one isolate will usually occupy several lines.

5.2 Data submission to and feedback from CAESAR

The CAESAR dataset (dataset with the file format required for CAESAR) is transferred to the CAESAR data manager on a quarterly or yearly basis. Please contact the CAESAR data manager to discuss the options for securely submitting the CAESAR dataset (caesar@rivm.nl).

The CAESAR data manager will upload the dataset into the CAESAR database platform. If the data cannot be uploaded to the CAESAR database because of a problem with the file, the CAESAR data manager will report back to the national data manager and, if possible, suggest a solution.

If the upload is successful, the following steps are performed.

- If multiple isolates from the same species in the same patient in the same calendar year are present in the dataset, all but the first isolate are removed. If on one and the same day a certain pathogen is isolated from both blood and CSF from a single patient, only data on the CSF isolate are included in analyses.

- If mandatory fields (please refer to Annex E) are missing, the record (isolate–antibiotic line) is rejected.

Once the CAESAR dataset is successfully uploaded into the CAESAR database, a feedback report is generated, which contains the following elements:

- technical details on the number of records received, rejected and accepted; each line containing the susceptibility test result of one antibiotic for one isolate is considered a record;

- an overview of unusual results, which could be rare resistance phenotypes or results that may indicate errors in AST or species identification;

- the number of isolates per pathogen, per laboratory, and in total; this overview may identify missing data for specific laboratories;

- the number and laboratory codes of laboratories that tested less than 75% of isolates for the antibiotics of interest, to provide insight into testing procedures followed in each laboratory and may indicate sequential or selective testing of antibiotics or identify missing data on specific antibiotic tests;

- demographic and clinical patient information, by pathogen and in total; the distribution (%) of isolate source and sex, age category and hospital department of patients is displayed, which gives an indication of the type of patients selected for sampling and may identify missing data on these characteristics; and

- resistance percentages for the CAESAR pathogen–antibiotic combinations and multidrug resistance.

The CAESAR data manager and epidemiologist check the feedback report. The report, and comments or questions that arose from the data, are sent back to the national AMR focal point and/or national data manager. Before approving the data, the AMR focal point and/or national data manager should assess the feedback report and answer any questions.

5.3 Creating the CAESAR dataset

The national data manager is responsible for preparing the CAESAR dataset from data collected at surveillance sites.

The first step is to extract surveillance data from the system in which they are collected. After the data extraction, the national data manager combines the datasets from all surveillance sites into one CAESAR dataset. Datasets may already meet the requirements for CAESAR; those that do not need to be converted, and the steps involved depend on the data entry method, which is discussed below.

5.3.1 Data entered into (electronic) IRFs

If a laboratory submits data on paper IRFs, the national data manager needs to digitalize the forms using e-IRFs, WHONET, or a LIS. Data that are directly entered into Microsoft Excel using eIRFs can be exported in the CAESAR format (a CSV text file). See Annex D for detailed information about using Microsoft Excel electronic IRFs.

5.3.2 Data from WHONET

Using an automated function, data can be exported directly from WHONET in the required CAESAR format. See the manual WHONET for CAESAR *(16)* for more information.

5.3.3 Data from a LIS

If AMR surveillance data are already in a database, for instance in a LIS, a query can be written to export the CAESAR dataset. In many cases the software company that designed the database or LIS can assist in composing this query.

If exporting a CAESAR dataset from the LIS is not technically possible, the LIS export functionality (query language) may be used to make a standard export from the LIS. The BacLink conversion utility (bundled with WHONET) can facilitate the transfer of data from the LIS into WHONET. Instructions are included in the manual WHONET for CAESAR *(16)*.

5.4 Data security and data anonymization

5.4.1 Data security

To guarantee continuous surveillance, data must not be lost due to computer malfunction, theft, or human errors. Regularly backing up files and saving them in a secure location such as a network-attached storage device or a local computer is therefore recommended. Another recommendation is to make a copy of the original file before editing, so that it is possible to go back to the original file if mistakes are made during editing.

5.4.2 Data anonymization

Laboratory data contain personal data. Disclosure of personal data – including patient identification – to anyone not involved in the patient's care constitutes a violation of patient–doctor confidentiality. It is therefore important that datasets sent to any surveillance programme, national or international, contain no information that may lead to the identification of a patient. Examples of identifying information are names, birth dates, home addresses, and national identifiers (e.g. social security numbers).

The CAESAR dataset submitted to the AMR surveillance group by each surveillance site should contain a unique code that does not contain any identifying information as a patient identifier. The national data manager should encrypt the patient identifiers prior to submitting the dataset to the CAESAR data manager. The electronic IRFs used by CAESAR provide an option to encrypt the patient identifier (see Annex D), as does WHONET (see the manual WHONET for CAESAR *(16)*). Surveillance sites can use encryption to create a unique patient identifier in case the original one contains identifying information.

The CAESAR data manager can give advice on encryption and provide tools (caesar@rivm.nl).

CHAPTER

6

Data analysis and reporting

RIVM analyses CAESAR data and prepares summary statistics for reporting. This chapter describes the methodology used for analysis and reporting.

6.1 Definitions and methods in CAESAR data analysis

6.1.1 Analysis results

Analysis results are presented as the percentage of tested isolates resistant (R) and susceptible, increased exposure (I) to an individual antibiotic or an antibiotic group.

6.1.2 Grouping of antibiotics

Resistance percentages can be presented for a single antibiotic and/or for a group of antibiotics. The priority sequence R → I → S is used to classify a pathogen. When combining the results for the antibiotics representing the group or class, the outcome is based on the most resistant result. For example, if a pathogen's susceptibility to imipenem is I and susceptibility to meropenem is R, then the susceptibility to imipenem/meropenem is set to R. Grouping of antibiotics for analyses is done only when the test characteristics for detecting resistance to all drugs within this group are comparable.

The full list of pathogen–antibiotic combinations under surveillance is in Table 2.

Table 2. Pathogen–antibiotic combinations under surveillance

Pathogen	Minimum panel to report to CAESAR	Antibiotic (group) reported in CAESAR analyses
E. coli (ESCCOL) and *K. pneumoniae* (KLEPNE)	AMP or AMX AMC TZP CTX or CRO CAZ GEN or TOB AMK CIP or LVX or OFX IPM or MEM ETP COL (MIC-based)	Ampicillin/amoxicillin (ESCCOL only) Amoxicillin-clavulanic acid Piperacillin-tazobactam Cefotaxime/ceftriaxone Ceftazidime Gentamicin/tobramycin Amikacin Ciprofloxacin/levofloxacin/ofloxacin Imipenem/meropenem Ertapenem Colistin
Salmonella spp. (SALSPP)	CTX or CRO CAZ CIP (MIC-based) or LVX IPM or MEM ETP	Cefotaxime/ceftriaxone Ceftazidime Ciprofloxacin/levofloxacin Imipenem/meropenem Ertapenem
P. aeruginosa (PSEAER)	TZP CAZ FEP GEN or TOB AMK CIP or LVX IPM or MEM COL (MIC-based)	Piperacillin-tazobactam Ceftazidime Cefepime Gentamicin/tobramycin Amikacin Ciprofloxacin/levofloxacin Imipenem/meropenem Colistin
Acinetobacter spp. (ACISPP)	GEN or TOB AMK CIP or LVX IPM or MEM COL (MIC-based)	Gentamicin/tobramycin Amikacin Ciprofloxacin/levofloxacin Imipenem/meropenem Colistin
S. aureus (STAAUR)	FOX (screen)* or OXA CIP or LVX or OFX VAN (MIC-based) LNZ RIF	MRSA Ciprofloxacin/levofloxacin/ofloxacin Vancomycin Linezolid Rifampicin
S pneumoniae (STRPNE)	OXA (screen) and PEN (MIC-based) if OXA=R ERY or CLR or AZM CTX or CRO (both MIC-based) LVX or MFX	Penicillin Erythromycin/clarithromycin/ azithromycin Cefotaxime/ceftriaxone Levofloxacin/moxifloxacin
E. faecalis (ENCFAE) and *E. faecium* (ENCFAI)	AMP or AMX GEH VAN LNZ	Ampicillin/amoxicillin High-level gentamicin Vancomycin Linezolid

AMC: amoxicillin–clavulanic acid; AMK: amikacin; AMP: ampicillin; AMX: amoxicillin; AZM: azithromycin; CAZ: ceftazidime; CIP: ciprofloxacin; CLR: clarithromycin; COL: colistin; CRO: ceftriaxone; CTX: cefotaxime; ERY: erythromycin; ETP: ertapenem; FEP: cefepime; FOX: cefoxitin; GEH: high-level gentamicin; GEN: gentamicin; IPM: imipenem; LNZ: linezolid; LVX: levofloxacin; MEM: meropenem; MFX: moxifloxacin; MIC: minimum inhibitory concentration; OFX: ofloxacin; OXA: oxacillin; PEN: penicillin; RIF: rifampicin; TOB: tobramycin; TZP: piperacillin-tazobactam; VAN: vancomycin.
*For methicillin-resistant *S. aureus* (MRSA) screening, cefoxitin (FOX) is the preferred method.

6.1.3 Calculating multidrug resistance

Resistance percentages for multidrug resistance are calculated, in addition to resistance percentages for individual antibiotics and antibiotic groups. Multidrug resistance is defined as resistance (R) to multiple

drugs, as listed in Table 3. Isolates with missing data on one or more of the required antibiotic groups are excluded from the analysis.

Table 3. Definitions of multidrug resistance by pathogen

Pathogen	Definition of multidrug resistance
E. coli (ESCCOL), *K. pneumoniae* (KLEPNE) and *Salmonella* spp. (SALSPP)	R to fluoroquinolones[1], third-generation cephalosporins[2], and aminoglycosides[3]
P. aeruginosa (PSEAER)	R to 3 or more antimicrobial groups among piperacillin + tazobactam, ceftazidime, aminoglycosides[3], fluoroquinolones[4], and carbapenems[5]
Acinetobacter spp. (ACISPP)	R to aminoglycosides[3], fluoroquinolones[4], and carbapenems[5]
S. pneumoniae (STRPNE)	I or R to penicillins[6] and R to macrolides[7]

[1] For *E. coli, K. pneumoniae*, and *Salmonella* spp., the fluoroquinolones group comprises ciprofloxacin, levofloxacin, and ofloxacin.
[2] The third-generation cephalosporins group comprises cefotaxime, ceftriaxone, and ceftazidime.
[3] The aminoglycosides group comprises gentamicin and tobramycin.
[4] For *P. aeruginosa* and *Acinetobacter* spp., the fluoroquinolones group comprises ciprofloxacin and levofloxacin.
[5] The carbapenems group comprises imipenem and meropenem.
[6] The penicillins group comprises penicillin or, if not available, oxacillin.
[7] The macrolides group comprises erythromycin, clarithromycin and azithromycin.

6.2 CAESAR publications

The WHO Regional Office for Europe annually publishes CAESAR results, including resistance data and country information on the progress being made related to national coordination of AMR activities, data quality control and surveillance. All CAESAR-related publications – including manuals, annual reports, the PoP protocol, and evaluation reports from PoP projects – are available for download from the WHO Regional Office for Europe AMR website *(7)*. Maps and graphs can also be accessed via the WHO European Health Information Gateway *(18)*.

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ANNEX

A

Laboratory and hospital questionnaires

Laboratory questionnaire 20___ (year)

(Please complete the questionnaire for each laboratory participating in CAESAR)

1. CAESAR laboratory code: (e.g. BR001)

2. Postal code of the laboratory:

3. City:

4. The total number of blood culture requests (sets)[1,2]:

5. CAESAR hospital codes for all hospitals served by the laboratory and, if available, the total number of blood culture requests (sets) per hospital served:

No.	CAESAR hospital code	Hospital-specific number of blood culture requests (sets)
1	(for example, BR001A)	
2	(for example, BR001B)	
3	(for example, BR001C)	
4		
5		
6		
7		

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1 One request/set consists of any number of blood culture bottles that are taken from one patient on a single occasion for diagnostic purposes.

2 If not available, please calculate by dividing the total number of blood culture bottles processed by the total number of bottles per blood culture request (set).

Hospital questionnaire 20__ (year)

(Please complete the questionnaire for each hospital participating in CAESAR)

1. CAESAR hospital code (for example BR001A):

2. CAESAR laboratory code (for example BR001):

3. Postal code of the hospital:

4. City of the hospital:

5. The level of care of the hospital : ☐ Primary level[3]
   (Important: check definitions) ☐ Secondary level[4]
   ☐ Tertiary level[5]
   ☐ Different, please specify:

6. Best estimate of catchment population of your hospital in 20__ (year)[6] (contact hospital administration):

7. Hospital size in beds in 20__ (year):

8. Number of intensive care beds in 20__ (year):

9. Total number of patient days[7] in the hospital in 20__ (year):
   OR (if not available)

10. The average occupancy rate in 20__ (year) (%):

11. Total number of patient admissions in 20__ (year):

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3 Primary level, often referred to as a district hospital or first-level referral. The hospital has few specialities, mainly internal medicine, obstetrics–gynaecology, paediatrics, and general surgery, or only general practice; limited laboratory services are available for general, but not for specialized pathological analysis; bed capacity ranges from 30 to 200 beds.

4 Secondary level often referred to as a provincial hospital. Highly differentiated by function with five to ten clinical specialities; bed capacity ranges from 200 to 800 beds.

5 Tertiary level often referred to as central, regional or tertiary-level hospital. Highly specialized staff and technical equipment, e.g. cardiology, ICU and specialized imaging units; clinical services are highly differentiated by function; may have teaching activities; bed capacity ranges from 300 to 1500 beds.

6 University/teaching hospitals may also serve as district hospitals, thereby actually serving two different populations. If this is the case for your hospital, please provide the catchment population for the university/tertiary care service.

7 Patient days: the number of patient days is the number of days spent in the institution for all patients occupying a bed. A day is measured at midnight, and the day of discharge is not counted as an extra day. This means that a patient admitted today and discharged tomorrow will have one patient day. Day patients will have zero patient days as they do not stay past midnight and must not be included in the total count.

ANNEX

B

Laboratory methods

Contents

1. Introduction

Virtually all pathogenic bacteria can acquire and express resistance to many of the antibiotics used for treatment. Assessment of resistance by means of antimicrobial susceptibility testing (AST) can be done once an organism is isolated and identified as the causative agent of an infection. Due to the emergence of antimicrobial resistance and spread of resistant pathogens, determination of antimicrobial susceptibility of bacteria is among the essential tasks of a clinical microbiology laboratory. Identification of the organism is not sufficient to help a physician choose the appropriate antibiotic treatment, thus physicians rely on laboratory AST reports listing the antibiotic options with which the organism can or cannot be treated.

Taking into account the resources of the laboratory, the target organisms and antibiotics available for prescription at the health care facility, each clinical laboratory should choose the AST method that best fits its needs. In order to produce reliable and reproducible AST results, clinical laboratories should only use standardized, internationally accepted AST methods. The methods used for AST of bacteria are diverse and include conventional methods such as broth microdilution and disk diffusion, as well as relatively novel methods such as gradient strips and semi-automated instruments for AST. Commercial AST systems provide important advantages such as decreased hands-on time, faster test results, testing against a wide array of antibiotics at once, automatic interpretation of results by the instrument, and integration of expert rules in the software. Nevertheless, there is still an ongoing interest in disk diffusion methodology. This is mainly due to the costs associated with consumables of commercial systems and the available systems' lack of flexibility. Generally, commercial systems test a fixed panel of antibiotics. Even though disk diffusion is among the oldest AST methods, organizations such as the Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) continuously work on developing and improving the methodology and provide yearly updates of their guidelines.

This annex focuses on the following topics:

- disk diffusion testing for pathogens for which susceptibility data is collected according to CAESAR methodology (section 2);

- quality control (QC) of disk diffusion testing (section 3);

- EUCAST expert rules (intrinsic resistance, exceptional resistance phenotypes, and interpretive rules) (section 4);

ANNEX B

- detection of important resistance mechanisms (section 5);

- final interpretation of AST results (section 6);

- rapid AST directly from blood culture bottles (section 7); and

- limitations of disk diffusion methodology (section 8).

The methodology for AST and QC testing with commercial systems is outside the scope of this annex. However, for the interpretation of results and confirmation of unexpected findings, the same EUCAST approach as outlined in sections 4–6 may be used.

In 2017, the external quality assessment (EQA) exercise in the CAESAR network showed that within the 248 participating laboratories, the disk diffusion method is the most widely used AST method (63%), followed by commercial, semi-automated instruments (36%), and MIC testing using gradient strip tests (1%) *(1)*. Furthermore, the 2017 EQA revealed that international guidelines are followed in all participating laboratories: CLSI (13%) and EUCAST (87%).

Disk diffusion methodology is a well-standardized method that meets the needs of clinical microbiology laboratories. However, the introduction of new agents, emergence of new resistance mechanisms, identification of new indications for existing antimicrobials, and change in available pharmacokinetic/pharmacodynamic data necessitate constant improvements in the methodology. Accordingly, EUCAST constantly reviews and updates its documents and tables. The tables and interpretive criteria in this annex should be regarded as exemplary, and readers are advised to refer to original sources on the EUCAST website *(2)* for further use. All EUCAST sources referred in this annex can be freely accessed online *(2)*.

2. Disk diffusion AST with EUCAST methodology

The basis of the EUCAST disk diffusion method is the minimum inhibitory concentration (MIC). Clinical MIC breakpoints are used to determine the susceptibility category of a given organism for the tested antimicrobial agent in question. The zone diameter breakpoints for EUCAST disk diffusion method are calibrated to reference MIC methods (e.g. broth microdilution and agar dilution). The EUCAST recommendations for MIC determination for non-fastidious organisms are in complete agreement with the recommendations from the International Standards Organization (ISO). EUCAST is preparing a document summarizing its methodology for MIC determination of non-fastidious and fastidious organisms. For the EUCAST disk diffusion method, however, readers are advised to refer to the "EUCAST Disk Diffusion Test Methodology" web page freely accessible on the EUCAST website *(3)*.

2.1 Media

Un-supplemented Mueller-Hinton (MH) agar is used for disk diffusion testing of non-fastidious organisms. For fastidious organisms, MH with 5% mechanically defibrinated horse blood and 20 mg/L ß-NAD[8] (Mueller-Hinton Fastidious, MH-F) is used (Table B.1). The ß-NAD component in MH-F should have a purity ≥98%.

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8   ß-Nicotinamide adenine dinucleotide

Table B.1. Testing conditions for CAESAR pathogens

Pathogen	Medium	Inoculum	Incubation conditions
Escherichia coli *Klebsiella pneumoniae* *Salmonella* spp. *Pseudomonas aeruginosa* *Acinetobacter* spp. *Staphylococcus aureus* *Enterococcus faecalis* *Enterococcus faecium*	MH agar[1]	0.5 McFarland	$35 \pm 1°C$ in air for 18 ± 2 hours (24 hours for glycopeptides with *Enterococcus* spp.)
Streptococcus pneumoniae	MH-F agar[2]	0.5 McFarland (if colonies grown on a blood agar plate are used for suspension preparation) 1 McFarland (if colonies grown on a chocolate agar plate are used for suspension preparation)	$35 \pm 1°C$ in 4–6% CO_2 for 18 ± 2 hours

CO_2: carbon dioxide.
[1] Mueller-Hinton agar.
[2] MH agar + 5% mechanically defibrinated horse blood + 20 mg/L ß-NAD.

2.1.1 Preparation of media

For the in-house preparation of media, the user should refer to the instructions provided by the respective manufacturer. For MH-F, only add the ß-NAD and blood components when the medium cools down to 42–45°C and then mix the contents well.

For standardization of the methodology, all agar plates should have a uniform depth of 4.0 ± 0.5 mm. This can only be achieved by pouring plates on a level surface and by distributing approximately 25 mL of molten agar into 90 mm circular plates or alternatively, 31 mL of molten agar into 100 mm circular plates *(4)*.

2.1.2 Storage of plates and issues to consider before use

The in-house prepared plates may be stored at 4–8°C. However, at the time of use, the plates should be at room temperature. Make sure that the surface of the agar is dry before use and that no drops of water are visible on the surface of the agar or inside the lid prior to inoculation, since the excess moisture may cause fuzzy zone edges and/or haze within zones. The plates may be dried at 20–25°C overnight or at 35°C for 15 minutes with the lid removed. If commercially prepared plates are used, store them according to manufacturers' recommendations.

2.2 Preparation of inoculum

Use a sterile loop or cotton swab to pick well-isolated colonies from an overnight growth on non-selective media. If possible, collect several morphologically similar colonies to avoid selecting an atypical variant. Prepare the suspension in saline and mix it to an even turbidity. Using a photometric device to measure the turbidity of the suspension is preferred. Adjust the density of the bacterial suspension to a 0.5 McFarland

standard, which approximately corresponds to the density of 1-2 x 10^8 colony forming units (CFUs)/mL for *E. coli*. A direct colony suspension method can be used to prepare the bacterial suspension in saline (0.85% solution of sodium chloride (NaCl) in water). For *S. pneumoniae*, if colonies grown on a chocolate agar plate are used for suspension preparation, the suspension should be matched to a 1 McFarland standard. The suspension should optimally be used within 15 minutes and always within 60 minutes after preparation.

2.3 Inoculation of agar plates

Use sterile cotton swabs to inoculate agar plates. Dip the swab into the suspension and then spread the inoculum evenly over the entire surface of the agar plate, either by swabbing in three directions or by using an automatic plate rotator. If using more than one agar plate for an organism, dip the cotton swab into the suspension before each plate.

To avoid over-inoculation of plates, remove excess fluid on the cotton swab by pressing and turning the swab against the inner wall of the suspension tube when testing Gram-negative organisms. For Gram-positive bacteria, however, do not remove the excess fluid.

Once the agar plates are inoculated with the bacterial suspension, apply the antimicrobial disks to the plates within 15 minutes of inoculation. Delaying the application of the disks and leaving the inoculated plates at room temperature may result in the organism starting to grow, which would lead to erroneous reduction in sizes of inhibition zones.

2.4 Application of antibiotic disks and incubation of plates

Using a disk with the correct disk content (also called disk "potency") is crucial. The EUCAST breakpoint tables and the QC tables *(5,6)* show the required disk contents. To prevent condensation, which leads to rapid deterioration of some agents, allow the disks to reach room temperature before opening cartridges or containers in which the disks are stored. Apply the disks firmly to the surface of the inoculated agar plate to ensure that they are in close and even contact with the agar surface. Since the initial diffusion of antimicrobial agents from disks is very fast, do not move disks once they are applied to the plate.

To avoid overlapping of inhibition zones, limit the number of disks on a plate. The maximum recommended number of disks on a 90 mm and 150 mm circular plates are 6 and 12, respectively.

To detect inducible clindamycin resistance, place the erythromycin and clindamycin disks at a distance of 12–20 mm from edge to edge for *S. aureus* and 12–16 mm from edge to edge for *S. pneumoniae*.

Some agents, including metronidazole, chloramphenicol and the fluoroquinolones, are inactivated by prolonged exposure to light and must therefore be protected from light. Some agents, e.g. amoxicillin-clavulanic acid, cefaclor and carbapenems, are more labile than others. For these, the in-use shelf life specified by the manufacturer might be shorter once the disk containers have been opened. To monitor the performance of antimicrobial disks, perform frequent QC testing as outlined in section 3.

Incubate the agar plates in an inverted position (upside down) within 15 minutes after disk application. Delaying the incubation of the plates and leaving plates at room temperature can lead to pre-diffusion which may result in erroneously large inhibition zones. Stacking plates too high in the incubator may affect results due to uneven heating. For most incubators, a maximum of five plates per stack is appropriate.

2.5 Examination of plates after incubation and reading of inhibition zone diameters

At the end of the incubation period (refer to "Incubation conditions" in Table B.1), remove the plates from the incubator and examine them as soon as possible. A correct inoculum and satisfactorily streaked plates should result in a confluent growth that is evenly distributed over the agar surface. The observation of individual colonies is an indication of suboptimal inoculated plates. In this case, repeat the test.

Read MH agar plates from the back with reflected light and the plate held above a dark background. Read MH-F agar plates from the front with the lid removed and with reflected light. (Do not use transmitted light by holding the plate towards the light source unless specifically instructed to do so. See the section on specific reading instructions below.)

Measure the inhibition zone diameters to the nearest millimetre with a ruler or a calliper, and read the zone edge at the point of complete inhibition as judged by the naked eye with the plate held about 30 cm from the eye.

Use the most recent EUCAST breakpoint tables to interpret the zone diameters into susceptibility categories.

Specific reading instructions are as follows.

- **Double zones**
  In case of double zones, or distinct colonies within zones, check the purity. In case of a mixed culture, repeat the test. For a pure culture, take into account colonies within zones when measuring the diameter.

- *Enterobacterales* **with ampicillin and amoxicillin-clavulanic acid**
  Ignore growth that may appear as a thin film producing an inner zone on some batches of MH agar.

- *S. aureus* **with cefoxitin**
  If cefoxitin disks are used to detect methicillin resistance in *S. aureus*, examine the zones carefully in good lighting to detect colonies within the zone of inhibition, which can be an indication of expression of heterogeneous methicillin resistance.

- *S. aureus* **with linezolid**
  Read linezolid zones from the back of the plate with the plate held up to light (transmitted light).

- *Enterococcus* **spp. with vancomycin**
  Examine vancomycin zone edges closely from the front of the plate with the plate held up to light (transmitted light). Fuzzy zone edges and colonies within zone indicate vancomycin resistance and require further investigation. Do not report isolates as susceptible before the 24-hour incubation period.

2.6 Supporting material for AST using disk diffusion

A checklist encompassing different stages of disk diffusion testing might be useful when first introducing the methodology in a laboratory (Table B.2).

Table B.2. Checklist for disk diffusion testing with EUCAST methodology

Step	Activity
Inoculum preparation	Organism was cultured on non-selective medium.
	Overnight (fresh) culture of the organism was used.
	The colonies were suspended in saline (0.85% NaCl solution in water).
	The density of the inoculum suspension was matched to 0.5 McFarland standard. (1 McFarland for *S. pneumoniae* grown on a chocolate agar plate)
	Inoculum suspension was used to inoculate the plates within 15 minutes after preparation.
Inoculation of Mueller-Hinton agar	The agar plate was at room temperature prior to inoculation.
	No water was visible on the agar surface or inside the lid.
	Plate was inoculated either by swabbing in three directions or by using a plate rotator.
	A sterile cotton swab was used to inoculate the plate.
	For Gram-negative organisms, the cotton swab was turned against the inside of the tube and excess fluid was removed to avoid over-inoculation.
	The inoculum was spread evenly over the entire agar surface and gaps between streaks were avoided.
Application of disks and incubation	The disks were allowed to reach room temperature before opening cartridges or containers.
	After application, the disks were in close and firm contact with the agar surface.
	The disks were applied within 15 minutes after the plate was inoculated.
	The plates were incubated within 15 minutes after disk application.
	The plates were incubated in inverted (upside down) position.
	MH agar plates were incubated in air for 18 ± 2 hours at 35 ± 1°C (24 hours for glycopeptides with *Enterococcus* spp.) MH-F agar plates were incubated in 4–6% CO_2 for 18 ± 2 hours at 35 ± 1°C.
Reading of zones	A confluent lawn of growth evenly distributed over the plate was obtained after the incubation.
	The zone edges were read at the point of complete inhibition of growth as judged by the naked eye.
	While reading, the plate was held about 30 cm from the eye.
	The inhibition zone diameter was measured to the nearest millimetre with a ruler or calliper.
	When reading, plate was illuminated with reflected light (for *S. aureus* with linezolid and *Enterococcus* spp. with vancomycin, plate was illuminated with transmitted light).
	MH agar plates were read from the back of the plate against a dark background. MH-F agar plates were read from the front of the plate, with the lid removed and with reflected light.

CO_2: carbon dioxide; NaCl: sodium chloride.

Instructional videos, prepared by EUCAST in collaboration with WHO, on how to perform AST using EUCAST recommended methods and interpretation are available online *(7)*. The EUCAST videos cover the following steps of AST:

1. preparation of inoculum

2. inoculation of agar plates for disk diffusion

3. application of antibiotic disks and incubation of plates

4. reading of inhibition zone diameters

5. guidance on the use of the breakpoint table.

The videos are narrated in English and are available with subtitles in several languages, including English and Russian *(7)*.

3. QC of disk diffusion testing

To monitor the performance of disk diffusion testing, use specific QC strains listed in the EUCAST QC tables (Table B.3) *(6)*. For QC purposes, strains with known susceptibility and strains with known resistance are used. Principal recommended strains are susceptible strains that are called "routine QC strains". The resistant strains are useful to confirm a laboratory`s ability to detect resistant organisms and these are called "extended QC strains".

Since it is not possible to control the β-lactamase inhibitor component of β-lactam-β-lactamase combination disks with the susceptible QC strains, a specific β-lactamase-producing strain is required to examine the β-lactamase inhibitor component. This is also considered part of routine QC.

The routine QC strains are used to monitor the performance of the laboratory and should be set up and checked daily, or at least four times per week, for antibiotics which are part of routine panels. The extended QC strains are complementary to the routine QC strains. These strains are recommended for detection of specific resistance mechanisms and are used to check that routine susceptibility will result in the correct susceptibility categorization. Extended QC should be performed with any change in the susceptibility testing system (with each new batch of disks or medium) and/or monthly.

Table B.3. Recommended routine and extended QC strains for EUCAST disk diffusion methodology for CAESAR pathogens

Pathogen	Routine QC	Extended QC
Escherichia coli *Klebsiella pneumoniae* *Salmonella* spp.	*E. coli* **ATCC 25922** (susceptible, wild-type) *E. coli* **ATCC 35218** (TEM-1 β-lactamase producer, ampicillin resistant – for control of the inhibitor component of β-lactam-β-lactamase inhibitor combinations)	*K. pneumoniae* **ATCC 700603** (SHV-18 ESBL-producer – for detection of ESBL production in *Enterobacterales*)
Pseudomonas aeruginosa	*P. aeruginosa* **ATCC 27853** (susceptible, wild-type) *E. coli* **ATCC 35218** (TEM-1 β-lactamase producer, ampicillin resistant – for control of the inhibitor component of β-lactam-β-lactamase inhibitor combinations)	NA
Acinetobacter spp.	*P. aeruginosa* **ATCC 27853** (susceptible, wild-type)	NA
Staphylococcus aureus	*S. aureus* **ATCC 29213** (weak β-lactamase producer)	*S. aureus* **NCTC 12493** (*mecA* positive, hetero-resistant MRSA – for detection of methicillin resistance in *S. aureus*)
Enterococcus faecalis *Enterococcus faecium*	*E. faecalis* **ATCC 29212** (susceptible, wild-type)	*E. faecalis* **ATCC 51299** (*vanB*-positive, high-level gentamicin and streptomycin resistant – for detection of *vanB*-mediated glycopeptide resistance and high-level aminoglycoside resistance in *Enterococcus* spp.)
Streptococcus pneumoniae	*S. pneumoniae* **ATCC 49619** (reduced susceptibility to penicillin)	NA

ESBL: extended-spectrum β-lactamase; NA: not available.

For the interpretation of the QC tests, EUCAST QC tables should be used that list targets as well as ranges for each antimicrobial agent *(6)*. As an example, inhibition zone diameter (mm) targets and ranges for *E. coli* ATCC 25922 are listed (Table B.4) for antimicrobial agents included in the Isolate Record Form (IRF) for *E. coli*.

Table B.4. Inhibition zone diameter (mm) target and range values for *Escherichia coli* ATCC 25922

Antimicrobial agent	Disk content (µg)	Inhibition zone diameter (mm)	
		Target	Range
Amikacin	30	22–23	19–26
Amoxicillin-clavulanic acid[1]	20–10	21	18–24[2]
Ampicillin	10	18–19	15–22
Cefotaxime	5	28	25–31
Ceftazidime	10	26	23–29
Ceftriaxone	30	32	29–35
Ciprofloxacin	5	33	29–37
Ertapenem	10	32–33	29–36
Gentamicin	10	22–23	19–26
Imipenem	10	29	26–32
Levofloxacin	5	33	29–37
Meropenem	10	31–32	28–35
Ofloxacin	5	31	29–33
Pefloxacin[3]	5	29	26–32
Piperacillin-tazobactam[1]	30–6	24	21–27
Tobramycin	10	22	18–26

[1] *E. coli* ATCC 35218 is used to check the inhibitor component.
[2] Ignore growth that may appear as a thin inner zone on some batches of Mueller-Hinton agar.
[3] For screening of ciprofloxacin resistance in *Salmonella* spp.

Repeated testing of EUCAST QC strains is expected to yield individual 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).

In addition to routine QC testing, test each new batch of MH agar to ensure that all zones are within range. Identify particular problems due to erroneous divalent cation, thymine and thymidine content of the MH agar by testing the following QC strains.

- *P. aeruginosa* ATCC 27853
  If the concentration of divalent cations (Ca^{2+}, Mg^{2+}) is high in the MH agar, aminoglycoside inhibition zones will result in values below QC limits.
  If the concentration of divalent cations (Ca^{2+}, Mg^{2+}) is low in the MH agar, aminoglycoside inhibition zones will result in values above QC limits.

- *E. faecalis* ATCC 29212

  If the thymine and thymidine concentration is higher than required, trimethoprim-sulfamethoxazole inhibition zones will result in values below QC limits.

The reference strains may be obtained from culture collections or commercial sources. In order to *preserve* the viability and characteristics of *the reference strains*, the strains should preferably be stored on beads at −70°C in glycerol broth. Non-fastidious strains can be stored at −20°C.

3.1 Interpretation of QC results

Before interpreting the results of clinical isolates, ensure that zone diameters for QC strains are within acceptable ranges.

Examine the results of the last 20 consecutive QC tests for trends (inhibition zones falling consistently above or below the target). Investigate if:

- two consecutive results of the last 20 tests are outside the acceptable ranges

- multiple results (>2) are out of range on the same day or

- resistance in a resistant QC strain is not detected.

In these cases, supress the susceptibility test results for clinical isolates, investigate the reason responsible for this deviation and repeat the tests. For a thorough investigation, consider the potential sources of errors listed in Table B.5.

Table B.5. Troubleshooting for errors in disk diffusion testing

Variable responsible for error	Potential source of error	Explanation
Medium	Storage of plates	Improper storage may lead to accumulation of excess moisture that can cause fuzzy zone edges and/or haze within zones.
	Instructions not followed when preparing plates	Medium should be cooled to 42–45°C before it is dispensed into sterile Petri dishes, and agar should be allowed to set before the plates are moved.
	Batch to batch variation	Some batches may exhibit problems due to erroneous content.
	Change of supplier of agar	Products from some manufacturers may include erroneous content.
	Supplements	Batch to batch variation, incorrect amount or expired product
	pH	pH should be within the range 7.2–7.4
	Agar depth and volume	Agar depth should be 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).
	Expiry date	Plates should be used within the expiry date.

Table B.5. Contd.

Variable responsible for error	Potential source of error	Explanation
Test conditions	The 15-15-15 minute rule not adhered to	Suspension should be used within 15 minutes; disks should be applied within 15 minutes after the plates are inoculated; incubation should start within 15 minutes after the disks are applied.
	Incubation conditions	Correct conditions for temperature, atmosphere and time should be followed.
	Incorrect inoculation	Too light, too heavy or uneven inoculation of plates
	Reading conditions	MH agar plates should be read from the back against a dark background illuminated with reflected light. MH-F agar plates should be read from the front with the lid removed illuminated with reflected light.
	Reading zone edges	Zone diameters should be measured to the nearest millimetre with a ruler or a calliper. Specific reading instructions should be followed for some pathogen–antibiotic combinations.
Disks	Incorrect disk	Wrong agent or wrong disk strength
	Disk potency	Incorrect storage, labile agent, expired disk
	Disks not at room temperature when containers opened	This is to avoid condensation, which leads to rapid deterioration of some agents.
	Too many disks on plate	Interference between agents or overlapping of zones.
Control organisms	Incorrect QC strain	Refer to EUCAST QC tables for correct QC strains.
	Mutation	Upon long-term storage or repeated subculturing, the strain may mutate and lose its characteristic features.
	Contamination	Contaminated QC strains cause erroneous results.
	Age of culture	Each week, the QC strain should be subcultured from the in-use vial onto appropriate non-selective media. From this culture, one subculture should be prepared on each day of the week. For fastidious organisms that will not survive on plates for five to six days, the strain should be subcultured daily for no more than one week.

3.2 Quality of antibiotic disks

A study conducted at the EUCAST Development Laboratory evaluated the quality of 16 strategically important antibiotic disks from nine manufacturers *(8)*. The study revealed some good and some poor quality among disks and manufacturers, highlighting wide variation in disk quality among manufacturers. These study results can be useful for laboratories in the process of deciding on which manufacturers' products to purchase or for other laboratories to evaluate the performance of their disks already in use.

4. EUCAST expert rules

Once AST has been performed, the results should be read, interpreted and validated. The increased complexity of antimicrobial resistance mechanisms and their clinical implications require an advanced level of knowledge to interpret AST results. Using the EUCAST clinical breakpoint tables, the inhibition zone diameters (in mm) can be categorized as susceptible, standard dosing regimen (S), susceptible, increased exposure (I) and resistant (R), which can then be communicated to clinicians. However, by considering the genetic basis of the resistance mechanism that reflects itself in a certain phenotype and by interpreting the results of different classes of antimicrobials together rather than interpreting each antimicrobial individually, it is possible to produce a more clinically relevant AST report. This practice uses the expert rules that describe actions to take on the basis of specific AST results. These actions can include recommendations on reporting, such as inferring susceptibility from one agent to other agents, suppressing results that may be inappropriate, and editing of results from S to I/R or from I to R on the basis of an inferred resistance mechanism *(9)*.

4.1 Intrinsic resistance and exceptional resistance phenotypes

The expert rules include the intrinsic resistances (which describe the natural resistance characteristic of all or almost all isolates of the bacterial species, as opposed to acquired and/or mutational resistance) and the exceptional resistance phenotypes (which describe the resistances that have not yet been reported or reported only very rarely) *(10)*. For isolates exhibiting an exceptional resistance phenotype, the identification and AST result must be confirmed and the isolate should be sent to a reference laboratory.

Enterobacterales, including *E. coli* and *K. pneumoniae*, are intrinsically resistant to benzylpenicillin, glycopeptides, fusidic acid, macrolides, lincosamides, streptogramins, rifampicin, daptomycin and linezolid. Non-fermentative Gram-negative bacteria (including *Acinetobacter* spp. and *P. aeruginosa*) are generally intrinsically resistant to benzylpenicillin, first- and second-generation cephalosporins, glycopeptides, fusidic acid, macrolides, lincosamides, streptogramins, rifampicin, daptomycin and linezolid. Gram-positive bacteria (including *S. aureus, Streptococcus* spp., *E. faecalis* and *E. faecium*) are intrinsically resistant to aztreonam, temocillin, polymyxin B/colistin and nalidixic acid.

Specific intrinsic resistance characteristics of CAESAR pathogens are listed in Table B.6. Exceptional resistance phenotypes of CAESAR pathogens are listed in Table B.7.

Table B.6. Intrinsic resistance in CAESAR pathogens

Pathogen	Intrinsically resistant to
E. coli	–
K. pneumoniae	Ampicillin
Acinetobacter spp.	Ampicillin, amoxicillin-clavulanic acid, cefazolin, cephalotin, cephalexin, cefadroxil, cefotaxime, ceftriaxone, aztreonam, ertapenem, trimethoprim, fosfomycin, tetracycline, doxycycline
P. aeruginosa	Ampicillin, amoxicillin-clavulanic acid, ampicillin-sulbactam, cefazolin, cefalothin, cephalexin, cefadroxil, cefotaxime, ceftriaxone, ertapenem, chloramphenicol, kanamycin, neomycin, trimethoprim, tetracyclines, tigecycline
S. aureus	Ceftazidime
S. pneumoniae	Fusidic acid, ceftazidime, aminoglycosides (low-level resistance)

Table B.6. Contd.

Pathogen	Intrinsically resistant to
E. faecalis	Fusidic acid, cephalosporins, aminoglycosides (low-level resistance), macrolides, clindamycin, quinupristin-dalfopristin, sulfonamides
E. faecium	Fusidic acid, cephalosporins, aminoglycosides (low-level resistance), macrolides, sulfonamides

Table B.7. Exceptional resistance phenotypes of CAESAR pathogens

Pathogen	Exceptional resistance phenotype
E. coli and *K. pneumoniae*	Resistant to colistin
Salmonella Typhi	Resistant to fluoroquinolones and/or carbapenems
P. aeruginosa and *Acinetobacter* spp.	Resistant to colistin
S. aureus	Resistant to vancomycin, teicoplanin, telavancin, dalbavancin, oritavancin, daptomycin, linezolid, tedizolid, quinupristin-dalfopristin and/or tigecycline
S. pneumoniae	Resistant to carbapenems, vancomycin, teicoplanin, telavancin, dalbavancin, oritavancin, daptomycin, linezolid, tedizolid, quinupristin-dalfopristin, tigecycline and/or rifampicin
Enterococcus spp.	Resistant to daptomycin, linezolid and/or tigecycline. Resistant to teicoplanin but not vancomycin
E. faecalis[1]	Resistant to ampicillin

[1] If susceptible to quinupristin-dalfopristin, consider misidentification. If also resistant to ampicillin, it is almost certainly *E. faecium*.

4.2 Interpretive rules

Expert rules, in general, have been developed to identify the underlying resistance mechanism and modify the results of AST when clinically required. This is accomplished by inference of resistance mechanisms from susceptibility test results, and interpretation of clinical susceptibility on the basis of the resistance mechanism. Examples of EUCAST interpretive rules are in Table B.8 *(9)*.

Table B.8. Examples of interpretive rules

Pathogen	Interpretive rule
Staphylococcus spp.	IF resistant in the cefoxitin screening test (MRSA), THEN report resistant to all beta-lactams, except those specifically licensed to treat infections caused by methicillin-resistant staphylococci expressing low affinity PBP2a; such agents must be tested individually. IF susceptible in the cefoxitin screening test (MSSA), THEN report as susceptible to all beta-lactams with recognised anti-staphylococcal activity.
S. pneumoniae	Rule for S. pneumoniae can be replaced with the below: IF resistant to erythromycin AND susceptible to clindamycin THEN test for inducible MLSB resistance; IF negative THEN report clindamycin susceptible; IF positive THEN report clindamycin resistant.
Enterobacterales except *Salmonella* spp.	IF resistant to ciprofloxacin, THEN report as resistant to all fluoroquinolones IF susceptible to ciprofloxacin, THEN report other fluoroquinolones as tested

Interpretation of AST results, along with expert rules, also serves as a QC measure since this practice enables the identification of unexpected findings (e.g. exceptional resistance phenotypes) and erroneous results (e.g. detection of organisms testing susceptible to antimicrobials for which they are intrinsically resistant to, which can be an indication of misidentification). Additionally, identification of the underlying resistance mechanisms helps to monitor the epidemiology of resistance mechanisms. As resistance mechanisms constantly evolve and the laboratory methods developed for their detection become more complex, the clinical microbiologist should follow the latest developments and implement expert rules in daily practice. This will not only increase the therapeutic success for patients but also help prevent the emergence of resistance.

5. Detection of important resistance mechanisms

Detection of some specific resistance mechanisms is particularly important for the reporting of AST results (i.e. determination of the susceptibility category), and for infection control and public health. EUCAST has published a special guideline that describes the methods for detection of important resistance mechanisms (10). This guideline includes nine important resistance mechanisms, and it summarizes recommended methods to detect the resistance mechanisms with an extensive list of references. Table B.9 shows the list of these nine resistance mechanisms of clinical and/or epidemiological importance.

In addition to the nine important resistance mechanisms, other clinically significant resistance mechanisms and their detection methods (e.g. inducible clindamycin resistance, low-level ciprofloxacin resistance in *Salmonella* spp.) are in the EUCAST clinical breakpoint table (5).

Table B.9. Antimicrobial resistance mechanisms of clinical and/or epidemiological importance

Resistance mechanism	Importance of detection of resistance mechanism		
	Required for clinical antimicrobial susceptibility categorization	Infection control purposes	Public health purposes
Carbapenemase-producing *Enterobacterales*	No	Yes	Yes
Extended-spectrum β-lactamase (ESBL)-producing *Enterobacterales*	No	Yes	Yes
Acquired AmpC β-lactamase-producing *Enterobacterales*	No	Yes	Yes
Polymyxin resistance in Gram-negative bacilli	Yes	Yes	Yes
Carbapenem resistance in *P. aeruginosa* and *Acinetobacter* spp.	No	Yes	Yes
Methicillin resistant *S. aureus* (MRSA)	Yes	Yes	Yes
Vancomycin resistant *S. aureus*	Yes	Yes	Yes
Vancomycin resistant *E. faecium* and *E. faecalis*	Yes	Yes	Yes
Penicillin non-wild-type *S. pneumoniae*	Yes	No	Yes

It is important to note that some resistance mechanisms (e.g. extended-spectrum β-lactamases or carbapenemases in *Enterobacterales*) do not always cause clinical resistance. However, the detection of resistance mechanism may be important for infection control and public health purposes.

6. Final interpretation of results

The inhibition zone diameters are translated into categories (S, I, R) to enable reporting to the clinicians. For this, EUCAST clinical breakpoint tables should be used in conjunction with other documents mentioned above. The expert rules (intrinsic resistances, exceptional resistance phenotypes and interpretive rules) should be considered, and if an important resistance mechanism is suspected, the required methodology should be followed.

In the beginning of 2019, EUCAST changed the definitions of the susceptibility categories. The updated definitions are listed in Table B.10.

Table B.10. New definitions of S, I and R susceptibility categories according to EUCAST

Category	Definition
S – Susceptible, standard dosing regimen	A microorganism is categorized as "Susceptible, standard dosing regimen" when there is a high likelihood of therapeutic success using a standard dosing regimen of the agent.
I – Susceptible, increased exposure[1]	A microorganism is categorized as "Susceptible, increased exposure"[1] when there is a high likelihood of therapeutic success because exposure to the agent is increased by adjusting the dosing regimen or by its concentration at the site of infection.
R – Resistant	A microorganism is categorized as "Resistant" when there is a high likelihood of therapeutic failure even when there is increased exposure.

[1] Exposure is a function of how the mode of administration, dose, dosing interval, infusion time, as well as distribution and excretion of the antimicrobial agent will influence the infecting pathogen at the site of infection.

With the introduction of new category definitions, EUCAST provides the following recommendations for the use of language in reporting and communicating regarding susceptibility categories.

- In laboratory AST reports, report isolates S, I or R.

- In communication with colleagues:

    - describe an isolate as belonging to the susceptibility category S, I or R;

    - refer to an isolate as susceptible (which includes S and I) or as resistant (R).

- For surveillance purposes, avoid merging categories – record as S, I and R. If merging does occur, never merge I and R, only S and I.

It is very important to emphasize that the updated definitions are related to agent exposure of the pathogen. This, in turn, is related to dose, dosing frequency (including going from repeated administration to intravenous infusion), dosing route, and to the pharmacokinetics of the agent and sometimes to the type of infection (urinary tract infections versus meningitis).

Dosing and modes of administration related to S, I and R of agents are available in the last tab of the EUCAST breakpoint table *(5)*.

Another important change in the EUCAST breakpoint table has been the introduction of the term Area of Technical Uncertainty (ATU) for occasions when a warning is required to alert the laboratory to the uncertainty of the AST result *(5)*. These warnings are typically required when inhibition zones or MICs of

resistant and susceptible pathogens overlap and the interpretation is uncertain. This usually occurs when variation in the method or in the interpretation is difficult to control. Starting from 2019, ATUs are listed in the EUCAST clinical breakpoint tables for various pathogen–antibiotic combinations. The pathogen–antibiotic combinations for which ATUs have been defined in the 2019 clinical breakpoint table, and are applicable to the CAESAR pathogens, are listed in Table B.11.

Table B.11. ATUs in *Enterobacterales*, *P. aeruginosa* and *S. aureus*

Pathogen	Antimicrobial	ATU	
		MIC	Zone diameter
Enterobacterales	Amoxicillin-clavulanic acid	No	Yes
	Piperacillin-tazobactam	Yes	Yes
	Ceftaroline	No	Yes
	Ciprofloxacin	Yes	Yes
P. aeruginosa	Piperacillin-tazobactam	No	Yes
	Ceftazidime-avibactam	No	Yes
	Colistin	Yes	NA
S. aureus	Ceftaroline	Yes	Yes
	Ceftobiprole	Yes	Yes
	Amikacin	Yes	Yes

NA: not applicable (colistin MIC determination should be performed with broth microdilution).

If the result falls in the ATU range, the laboratory can take several actions.

- **Repeat the test** – only if there is reason to suspect a technical error.

- **Perform an alternative test** (determine an MIC, perform a molecular test or a test to determine the resistance mechanism).

- **Report results in the ATU as "uncertain"**, which can be achieved by leaving the interpretation blank and adding a comment.

- **Report results in the ATU as "R".** If there are several good alternatives in the AST report this may be the easiest and safest option.

- Take the opportunity to **discuss the results with the clinician.**

For results that fall in the ATU range, a trustworthy categorization should be sought if:

- only a few antibiotics are available to the clinician

- the pathogen is isolated from a blood culture

- an alternative method could be helpful without a delay.

An explanatory video on the updated definitions of S, I and R and on the use of the ATU are available online at the EUCAST website *(12)*.

7. Rapid AST directly from blood culture bottles

In 2018, EUCAST published recommendations for a new methodology that enables disk diffusion AST with short incubation periods (4, 6 and 8 hours) directly from blood culture bottles *(13)*. The proposed methodology is simple and no centrifugation or dilution of the inoculum is required. The agar plates (MH, MH-F) are inoculated using 100–150 μL of blood directly from a positive blood culture, and interpreted after shortened incubation (4, 6 and 8 hours) with breakpoints adapted to each incubation time. Only zone diameters with an obvious zone edge should be read after 4 hours, otherwise the plates should be re-incubated and read after 6 or 8 hours. For the interpretation of results, use breakpoints validated specifically for rapid AST method *(14)*.

If satisfactory growth is not achieved after 8 hours, incubation cannot be prolonged – the method is only validated for the short incubation time. If the result cannot be interpreted after 8 hours of incubation, re-test the isolate with the EUCAST standard disk diffusion method.

An important difference from standard disk diffusion methodology is that the plates (including MH agar) are read from the front, with the lid removed. Since the EUCAST breakpoint table for the rapid AST is specific to this method, do not use the regular clinical breakpoint tables with this method.

The rapid AST method is currently validated for the following species:

- *E. coli*

- *K. pneumoniae*

- *P. aeruginosa*

- *A. baumannii*

- *S. aureus*

- *S. pneumoniae*

- *E. faecalis*

- *E. faecium.*

Since this method relies on correct identification of species, identity of species must be known prior to interpretation of AST results.

8. Limitations of disk diffusion methodology

Even though disk diffusion is the preferred method in many laboratories, some pathogen–antibiotic combinations cannot be tested with the disk diffusion method; in other situations an MIC is required to infer susceptibility to one/multiple antimicrobial(s) (Table B.12).

Table B.12. Examples of when MIC determination is required

Pathogen	Antimicrobial	Rationale
Enterobacterales *P. aeruginosa* *Acinetobacter* spp.	Colistin	Colistin MIC determination should be performed with broth microdilution. Disk diffusion cannot be used for susceptibility testing of colistin. It does not discriminate between susceptible and resistant isolates. Currently available gradient strip tests underestimate colistin MIC values and underestimate resistance and should be avoided, even when QC results are within range.
S. aureus	Vancomycin	Disk diffusion is unreliable and cannot distinguish between wild-type isolates and those with non-*vanA*-mediated glycopeptide resistance. Glycopeptide MICs are method-dependent and should be determined by broth microdilution (ISO standard 20776-1).
S. pneumoniae	Beta-lactams (if oxacillin zone diameter < 20 mm)	Benzylpenicillin: For infections other than meningitis, determine the MIC and interpret according to the clinical breakpoints. For meningitis, report resistant. Phenoxymethylpenicillin: For all indications, report resistant. Oxacillin zone diameter ≥8 mm For ampicillin, amoxicillin and piperacillin (without and with beta-lactamase inhibitor), cefepime, cefotaxime and ceftriaxone: report susceptible for other indications, but in meningitis the MIC for the agent considered for clinical use should be determined. Oxacillin zone diameter <8 mm For cefepime, cefotaxime and ceftriaxone: determine the MIC.

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ANNEX

C

Isolate record forms

Isolate record forms provide a convenient method to collect diagnostic AST results for surveillance. The forms below cover the minimum panel of antibiotics for CAESAR reporting. These forms serve as a template and can be adapted to fit the national surveillance system guidelines. Because the CAESAR minimum panel of antibiotics in some cases contains several equivalent antibiotics (e.g. cefotaxime or ceftriaxone), countries can choose which antibiotic to use according to national guidelines. The redundant antibiotics, those that will not be tested in the laboratory, can be removed from the isolate record forms. Likewise, the isolate record forms can be extended with additional antibiotics that are important for local or national surveillance (e.g. trimethoprim-sulfamethoxazole or fosfomycin).

The data from these isolate record forms should be digitalized (e.g. in the Excel-based electronic isolate record form (eIRF) or in WHONET) before submission to CAESAR.

Isolate Record Form *E. coli*

To be filled out by the laboratory

Instructions: Please send data of the first **blood and/or cerebrospinal fluid (CSF)** isolate of all patients with an invasive *E. coli* infection. Use 1 form per isolate.

Laboratory Code		
Isolate ID	Isolate source ☐ Blood ☐ CSF	Date of sample collection (yyyy-mm-dd)
Patient ID	Gender ☐ Male ☐ Female ☐ Other ☐ Unknown	Year of birth (yyyy)
Hospital ID	Origin of patient ☐ Inpatient ☐ Outpatient ☐ Other ☐ Unknown	Date of admission (yyyy-mm-dd)

Hospital Department

☐ Internal medicine ☐ Paediatrics/neonatal ☐ Paediatrics/neonatal ICU ☐ Surgery ☐ Haematology/Oncology
☐ Obstetrics/Gynaecology ☐ Intensive care unit (ICU) ☐ Emergency department ☐ Urology ☐ Infectious disease
☐ Other ☐ Unknown

Antibiotic susceptibility testing (S/I/R, zone **and/or** MIC) MIC can be done by broth dilution or gradient strip test

Antibiotic	SIR (final interpretation result of all different susceptibility test performed) Fill in S, I or R	Zone diameter (mm)	Zone diameter interpretation Fill in S, I or R	MIC Automated system (mg/L)	MIC interpretation Automated system Fill in S, I or R	MIC Gradient strip test/ agar dilution/ microdilution (mg/L)	MIC interpretation Gradient strip test/agar dilution/micro-dilution Fill in S, I or R
Ampicillin	☐	☐☐	☐	- - - - - -	☐	- - - - - -	☐
or Amoxicillin	☐	–	–	- - - - - -	☐	- - - - - -	☐
Amoxicillin-clavulanic acid*	☐	☐☐	☐	- - - - - -	☐	- - - - - -	☐
Piperacillin-tazobactam	☐	☐☐	☐	- - - - - -	☐	- - - - - -	☐
Cefotaxime	☐	☐☐	☐	- - - - - -	☐	- - - - - -	☐
or Ceftriaxone	☐	☐☐	☐	- - - - - -	☐	- - - - - -	☐
Ceftazidime	☐	☐☐	☐	- - - - - -	☐	- - - - - -	☐
Gentamicin	☐	☐☐	☐	- - - - - -	☐	- - - - - -	☐
or Tobramycin	☐	☐☐	☐	- - - - - -	☐	- - - - - -	☐
Amikacin	☐	☐☐	☐	- - - - - -	☐	- - - - - -	☐
Ciprofloxacin	☐	☐☐	☐	- - - - - -	☐	- - - - - -	☐
or Ofloxacin	☐	☐☐	☐	- - - - - -	☐	- - - - - -	☐
or Levofloxacin	☐	☐☐	☐	- - - - - -	☐	- - - - - -	☐
Imipenem**	☐	☐☐	☐	- - - - - -	☐	- - - - - -	☐
or Meropenem**	☐	☐☐	☐	- - - - - -	☐	- - - - - -	☐
Ertapenem	☐	☐☐	☐	- - - - - -	☐	- - - - - -	☐
Colistin***	☐	–	–	–	–	- - - - - -	☐

Resistance mechanism test
ESBL (extended-spectrum ß-lactamase) ☐ positive ☐ negative ☐ unknown
Carbapenemase ☐ positive ☐ negative ☐ unknown

*According to EUCAST amoxicillin-clavulanic acid zone diameter and MIC value are interpreted twice: 1. to report for uncomplicated urinary tract infections (UTIs), 2. to report basic sensitivity for all the other infections. For reporting to CAESAR use basic breakpoints (not the UTI breakpoints).

**If imipenem or meropenem are the carbapenems tested, the same zone diameter or MIC value is interpreted twice: 1. to report susceptibility, 2. to screen for possible carbapenemase producers. For reporting to CAESAR use clinical breakpoints (not the screen breakpoints).

***Colistin susceptibility should be determined by broth microdilution.

Send this form to (Name/Institute/Contact details):

Isolate Record Form *K. pneumoniae*

To be filled out by the laboratory

Instructions: Please send data of the first **blood and/or cerebrospinal fluid (CSF)** isolate of all patients with an invasive *K. pneumoniae* infection. Use 1 form per isolate.

Laboratory Code		
Isolate ID	Isolate source ☐ Blood ☐ CSF	Date of sample collection (yyyy-mm-dd)
Patient ID	Gender ☐ Male ☐ Female ☐ Other ☐ Unknown	Year of birth (yyyy)
Hospital ID	Origin of patient ☐ Inpatient ☐ Outpatient ☐ Other ☐ Unknown	Date of admission (yyyy-mm-dd)

Hospital Department

☐ Internal medicine ☐ Paediatrics/neonatal ☐ Paediatrics/neonatal ICU ☐ Surgery ☐ Haematology/Oncology
☐ Obstetrics/Gynaecology ☐ Intensive care unit (ICU) ☐ Emergency department ☐ Urology ☐ Infectious disease
☐ Other ☐ Unknown

Antibiotic susceptibility testing (S/I/R, zone **and/or** MIC) MIC can be done by broth dilution or gradient strip test

Antibiotic	SIR (final interpretation result of all different susceptibility test performed) Fill in S, I or R	Zone diameter (mm)	Zone diameter interpretation Fill in S, I or R	MIC Automated system (mg/L)	MIC interpretation Automated system Fill in S, I or R	MIC Gradient strip test/ agar dilution/ microdilution (mg/L)	MIC interpretation Gradient strip test/agar dilution/micro-dilution Fill in S, I or R
Amoxicillin-clavulanic acid*	☐	☐☐	☐	- - - - - -	☐	- - - - - -	☐
Piperacillin-tazobactam	☐	☐☐	☐	- - - - - -	☐	- - - - - -	☐
Cefotaxime	☐	☐☐	☐	- - - - - -	☐	- - - - - -	☐
or Ceftriaxone	☐	☐☐	☐	- - - - - -	☐	- - - - - -	☐
Ceftazidime	☐	☐☐	☐	- - - - - -	☐	- - - - - -	☐
Gentamicin	☐	☐☐	☐	- - - - - -	☐	- - - - - -	☐
or Tobramycin	☐	☐☐	☐	- - - - - -	☐	- - - - - -	☐
Amikacin	☐	☐☐	☐	- - - - - -	☐	- - - - - -	☐
Ciprofloxacin	☐	☐☐	☐	- - - - - -	☐	- - - - - -	☐
or Ofloxacin	☐	☐☐	☐	- - - - - -	☐	- - - - - -	☐
or Levofloxacin	☐	☐☐	☐	- - - - - -	☐	- - - - - -	☐
Imipenem**	☐	☐☐	☐	- - - - - -	☐	- - - - - -	☐
or Meropenem**	☐	☐☐	☐	- - - - - -	☐	- - - - - -	☐
Ertapenem	☐	☐☐	☐	- - - - - -	☐	- - - - - -	☐
Colistin***	☐	-	-	-	-	- - - - - -	☐

Resistance mechanism test
ESBL (extended-spectrum ß-lactamase)   ☐ positive ☐ negative ☐ unknown

Carbapenemase   ☐ positive ☐ negative ☐ unknown

* According to EUCAST amoxicillin-clavulanic acid zone diameter and MIC value are interpreted twice: 1. to report for uncomplicated urinary tract infections (UTIs), 2. to report basic sensitivity for all the other infections. For reporting to CAESAR use basic breakpoints (not the UTI breakpoints).

** If imipenem or meropenem are the carbapenems tested, the same zone diameter or MIC value is interpreted twice: 1. to report susceptibility, 2. to screen for possible carbapenemase producers. For reporting to CAESAR use clinical breakpoints (not the screen breakpoints).

*** Colistin susceptibility should be determined by broth microdilution.

Send this form to (Name/Institute/Contact details):

Isolate Record Form *Salmonella* spp.

To be filled out by the laboratory

Instructions: Please send data of the first **blood and/or cerebrospinal fluid (CSF)** isolate of all patients with an invasive *Salmonella* infection. Use 1 form per isolate.

Laboratory Code		
Isolate ID	Isolate source ☐ Blood ☐ CSF	Date of sample collection (yyyy-mm-dd)
Patient ID	Gender ☐ Male ☐ Female ☐ Other ☐ Unknown	Year of birth (yyyy)
Hospital ID	Origin of patient ☐ Inpatient ☐ Outpatient ☐ Other ☐ Unknown	Date of admission (yyyy-mm-dd)

Hospital Department

☐ Internal medicine ☐ Paediatrics/neonatal ☐ Paediatrics/neonatal ICU ☐ Surgery ☐ Haematology/Oncology
☐ Obstetrics/Gynaecology ☐ Intensive care unit (ICU) ☐ Emergency department ☐ Urology ☐ Infectious disease
☐ Other ☐ Unknown

Antibiotic susceptibility testing (S/I/R, zone **and/or** MIC) MIC can be done by broth dilution or gradient strip test

Antibiotic	SIR (final interpretation result of all different susceptibility test performed) Fill in S, I or R	Zone diameter (mm)	Zone diameter interpretation Fill in S, I or R	MIC Automated system (mg/L)	MIC interpretation Automated system Fill in S, I or R	MIC Gradient strip test/ agar dilution/ microdilution (mg/L)	MIC interpretation Gradient strip test/agar dilution/micro-dilution Fill in S, I or R
Cefotaxime	☐	☐☐	☐	- - - - - -	☐	- - - - - -	☐
or Ceftriaxone	☐	☐☐	☐	- - - - - -	☐	- - - - - -	☐
Ceftazidime	☐	☐☐	☐	- - - - - -	☐	- - - - - -	☐
Ciprofloxacin	☐	☐☐	☐	- - - - - -	☐	- - - - - -	☐
or Levofloxacin	☐	☐☐	☐	- - - - - -	☐	- - - - - -	☐
Pefloxacin screen*	☐	☐☐	☐	–	–	–	–
Imipenem**	☐	☐☐	☐	- - - - - -	☐	- - - - - -	☐
or Meropenem**	☐	☐☐	☐	- - - - - -	☐	- - - - - -	☐
Ertapenem	☐	☐☐	☐	- - - - - -	☐	- - - - - -	☐

Resistance mechanism test

ESBL (extended-spectrum β-lactamase) ☐ positive ☐ negative ☐ unknown

Carbapenemase ☐ positive ☐ negative ☐ unknown

*   Do not report pefloxacin to clinicians. Pefloxacin disk is used as a screening disk. If pefloxacin S report ciprofloxacin S, if pefloxacin R report ciprofloxacin R.
** If imipenem or meropenem are the carbapenems tested, the same zone diameter or MIC value is interpreted twice: 1. to report susceptibility, 2. to screen for possible carbapenemase producers. For reporting to CAESAR use clinical breakpoints (not the screen breakpoints)

Send this form to (Name/Institute/Contact details):

Isolate Record Form *P. aeruginosa*

To be filled out by the laboratory

Instructions: Please send data of the first **blood and/or cerebrospinal fluid (CSF)** isolate of all patients with an invasive *P. aeruginosa* infection. Use 1 form per isolate.

Laboratory Code		
Isolate ID	Isolate source ☐ Blood ☐ CSF	Date of sample collection (yyyy-mm-dd)
Patient ID	Gender ☐ Male ☐ Female ☐ Other ☐ Unknown	Year of birth (yyyy)
Hospital ID	Origin of patient ☐ Inpatient ☐ Outpatient ☐ Other ☐ Unknown	Date of admission (yyyy-mm-dd)

Hospital Department

☐ Internal medicine ☐ Paediatrics/neonatal ☐ Paediatrics/neonatal ICU ☐ Surgery ☐ Haematology/Oncology
☐ Obstetrics/Gynaecology ☐ Intensive care unit (ICU) ☐ Emergency department ☐ Urology ☐ Infectious disease
☐ Other ☐ Unknown

Antibiotic susceptibility testing (S/I/R, zone **and/or** MIC) MIC can be done by broth dilution or gradient strip test

Antibiotic	SIR (final interpretation result of all different susceptibility test performed) Fill in S, I or R	Zone diameter (mm)	Zone diameter interpretation Fill in S, I or R	MIC Automated system (mg/L)	MIC interpretation Automated system Fill in S, I or R	MIC Gradient strip test/ agar dilution/ microdilution (mg/L)	MIC interpretation Gradient strip test/agar dilution/micro-dilution Fill in S, I or R
Piperacillin-tazobactam	☐	☐ ☐	☐	- - - - - -	☐	- - - - - -	☐
Ceftazidime	☐	☐ ☐	☐	- - - - - -	☐	- - - - - -	☐
Cefepime	☐	☐ ☐	☐	- - - - - -	☐	- - - - - -	☐
Gentamicin or Tobramycin	☐ ☐	☐ ☐ ☐ ☐	☐ ☐	- - - - - - - - - - - -	☐ ☐	- - - - - - - - - - - -	☐ ☐
Amikacin	☐	☐ ☐	☐	- - - - - -	☐	- - - - - -	☐
Ciprofloxacin or Levofloxacin	☐ ☐	☐ ☐ ☐ ☐	☐ ☐	- - - - - - - - - - - -	☐ ☐	- - - - - -	☐ ☐
Imipenem or Meropenem	☐ ☐	☐ ☐ ☐ ☐	☐ ☐	- - - - - - - - - - - -	☐ ☐	- - - - - - - - - - - -	☐ ☐
Colistin*	☐	–	–	–	–	- - - - - -	☐

* Colistin susceptibility should be determined by broth microdilution.

Send this form to (Name/Institute/Contact details):

Isolate Record Form *Acinetobacter* spp.

To be filled out by the laboratory

Instructions: Please send data of the first **blood and/or cerebrospinal fluid (CSF)** isolate of all patients with an invasive *Acinetobacter* spp. infection. Use 1 form per isolate.

Laboratory Code		
Isolate ID	Isolate source ☐ Blood ☐ CSF	Date of sample collection (yyyy-mm-dd)
Patient ID	Gender ☐ Male ☐ Female ☐ Other ☐ Unknown	Year of birth (yyyy)
Hospital ID	Origin of patient ☐ Inpatient ☐ Outpatient ☐ Other ☐ Unknown	Date of admission (yyyy-mm-dd)

Hospital Department

☐ Internal medicine ☐ Paediatrics/neonatal ☐ Paediatrics/neonatal ICU ☐ Surgery ☐ Haematology/Oncology
☐ Obstetrics/Gynaecology ☐ Intensive care unit (ICU) ☐ Emergency department ☐ Urology ☐ Infectious disease
☐ Other ☐ Unknown

Antibiotic susceptibility testing (S/I/R, zone **and/or** MIC) MIC can be done by broth dilution or gradient strip test

Antibiotic	SIR (final interpretation result of all different susceptibility test performed) Fill in S, I or R	Zone diameter (mm)	Zone diameter interpretation Fill in S, I or R	MIC Automated system (mg/L)	MIC interpretation Automated system Fill in S, I or R	MIC Gradient strip test/ agar dilution/ microdilution (mg/L)	MIC interpretation Gradient strip test/agar dilution/micro-dilution Fill in S, I or R
Gentamicin	☐	☐☐	☐	- - - - - -	☐	- - - - - -	☐
or Tobramycin	☐	☐☐	☐	- - - - - -	☐	- - - - - -	☐
Amikacin	☐	☐☐	☐	- - - - - -	☐	- - - - - -	☐
Ciprofloxacin	☐	☐☐	☐	- - - - - -	☐	- - - - - -	☐
or Levofloxacin	☐	☐☐	☐	- - - - - -	☐	- - - - - -	☐
Imipenem	☐	☐☐	☐	- - - - - -	☐	- - - - - -	☐
or Meropenem	☐	☐☐	☐	- - - - - -	☐	- - - - - -	☐
Colistin*	☐	–	–	–	–	- - - - - -	☐

*   Colistin susceptibility should be determined by broth microdilution.

Send this form to (Name/Institute/Contact details):

Isolate Record Form *S. aureus*

To be filled out by the laboratory

Instructions: Please send data of the first **blood and/or cerebrospinal fluid (CSF)** isolate of all patients with an invasive *S. aureus* infection. Use 1 form per isolate.

Laboratory Code		
Isolate ID	Isolate source ☐ Blood ☐ CSF	Date of sample collection (yyyy-mm-dd)
Patient ID	Gender ☐ Male ☐ Female ☐ Other ☐ Unknown	Year of birth (yyyy)
Hospital ID	Origin of patient ☐ Inpatient ☐ Outpatient ☐ Other ☐ Unknown	Date of admission (yyyy-mm-dd)

Hospital Department

☐ Internal medicine ☐ Paediatrics/neonatal ☐ Paediatrics/neonatal ICU ☐ Surgery ☐ Haematology/Oncology
☐ Obstetrics/Gynaecology ☐ Intensive care unit (ICU) ☐ Emergency department ☐ Urology ☐ Infectious disease
☐ Other ☐ Unknown

Antibiotic susceptibility testing (S/I/R, zone **and/or** MIC) MIC can be done by broth dilution or gradient strip test

Antibiotic	SIR (final interpretation result of all different susceptibility test performed) Fill in S, I or R	Zone diameter (mm)	Zone diameter interpretation Fill in S, I or R	MIC Automated system (mg/L)	MIC interpretation Automated system Fill in S, I or R	MIC Gradient strip test/ agar dilution/ microdilution (mg/L)	MIC interpretation Gradient strip test/agar dilution/micro-dilution Fill in S, I or R
Cefoxitin screen*	☐	☐☐	☐	- - - - - -	☐	- - - - - -	☐
or Oxacillin	☐	–	–	- - - - - -	☐	- - - - - -	☐
Ciprofloxacin	☐	☐☐	☐	- - - - - -	☐	- - - - - -	☐
or Levofloxacin	☐	☐☐	☐	- - - - - -	☐	- - - - - -	☐
or Ofloxacin	☐	☐☐	☐	- - - - - -	☐	- - - - - -	☐
Norfloxacin screen**	☐	☐☐	☐	–	–	–	–
Vancomycin	☐	–	–	- - - - - -	☐	- - - - - -	☐
Linezolid	☐	☐☐	☐	- - - - - -	☐	- - - - - -	☐
Rifampicin	☐	☐☐	☐	- - - - - -	☐	- - - - - -	☐

* For MRSA screening, cefoxitin is the preferred method. For clinicians cefoxitin/oxacillin data should be reported as data for semisynthetic penicillin locally available (cloxacillin, flucloxacillin, etc.)

** Do not report norfloxacin to clinicians. Norfloxacin disk is used as a screening disk. If norfloxacin S: you can report ciprofloxacin, ofloxacin, levofloxacin and moxifloxacin S. If norfloxacin R: you have to test for susceptibility to each quinolone individually.

Send this form to (Name/Institute/Contact details):

Isolate Record Form *S. pneumoniae*

To be filled out by the laboratory

Instructions: Please send data of the first **blood and/or cerebrospinal fluid (CSF)** isolate of all patients with an invasive *S. pneumoniae* infection. Use 1 form per isolate.

Laboratory Code		
Isolate ID	Isolate source ☐ Blood ☐ CSF	Date of sample collection (yyyy-mm-dd)
Patient ID	Gender ☐ Male ☐ Female ☐ Other ☐ Unknown	Year of birth (yyyy)
Hospital ID	Origin of patient ☐ Inpatient ☐ Outpatient ☐ Other ☐ Unknown	Date of admission (yyyy-mm-dd)

Hospital Department

☐ Internal medicine   ☐ Paediatrics/neonatal   ☐ Paediatrics/neonatal ICU   ☐ Surgery   ☐ Haematology/Oncology
☐ Obstetrics/Gynaecology   ☐ Intensive care unit (ICU)   ☐ Emergency department   ☐ Urology   ☐ Infectious disease
☐ Other   ☐ Unknown

Antibiotic susceptibility testing (S/I/R, zone **and/or** MIC) MIC can be done by broth dilution or gradient strip test

Antibiotic	SIR (final interpretation result of all different susceptibility test performed) Fill in S, I or R	Zone diameter (mm)	Zone diameter interpretation Fill in S, I or R	MIC Automated system (mg/L)	MIC interpretation Automated system Fill in S, I or R	MIC Gradient strip test/ agar dilution/ microdilution (mg/L)	MIC interpretation Gradient strip test/agar dilution/micro-dilution Fill in S, I or R
Penicillin*	☐	–	–	- - - - - -	☐	- - - - - -	☐
Oxacillin screen**	☐	☐☐	☐	–	–	–	–
Ampicillin***	☐	☐☐	☐	- - - - - -	☐	- - - - - -	☐
Amoxicillin oral***	☐	–	☐	- - - - - -	☐	- - - - - -	☐
Erythromycin****	☐	☐☐	☐	- - - - - -	☐	- - - - - -	☐
or Clarithromycin	☐	–	–	- - - - - -	☐	- - - - - -	☐
or Azithromycin	☐	–	–	- - - - - -	☐	- - - - - -	☐
Cefotaxime	☐	–	–	- - - - - -	☐	- - - - - -	☐
or Ceftriaxone	☐	–	–	- - - - - -	☐	- - - - - -	☐
Norfloxacin screen*****	☐	☐☐	☐	–	–	–	–
Levofloxacin	☐	☐☐	☐	- - - - - -	☐	- - - - - -	☐
or Moxifloxacin	☐	☐☐	☐	- - - - - -	☐	- - - - - -	☐
Serotype:							

*   For oxacillin R isolates test penicillin MIC: ≤0.06 (S): 0.125–2.0 (I); ≥2.0 (R) or send the isolate to the Reference laboratory

**   For surveillance purposes enter data for oxacillin; for reporting to clinicians enter data for PENICILLIN: if oxacillin S report penicillin S, if oxacillin R report oral penicillin R + note: for infections not involving central nervous system high dose parenteral penicillin may be used for isolates with MIC ≤ 2.0 mg/L. For oxacillin R isolates perform penicillin and ceftriaxone or cefotaxime MIC (see below)

***   Results for ampicillin and amoxicillin oral can be derived from one test:
if disk diffusion is done using 2 μg ampicillin disk: zone diameter interpretation is ≥22 (S), 16–21 (I), <16 (R) for ampicillin and ≥22 (S), 19–21 (I), <19 (R) for amoxicillin oral if MIC gradient/dilution test is done: MIC interpretation is ≤0.5 (S), 1.0–2.0 (I), >2.0 (R) for ampicillin and ≤0.5 (S), 1.0 (I), >1.0 (R) for amoxicillin oral

**** Erythromycin is a class representative for clarithromycin and azithromycin

***** Do not report norfloxacin to clinicians. Norfloxacin disk is used as a screening disk. If norfloxacin S: you can report levofloxacin S and moxifloxacin S. If norfloxacin R: you have to test for susceptibility to levofloxacin and moxifloxacin individually.

Send this form to (Name/Institute/Contact details):

Isolate Record Form ☐ *E. faecium* ☐ *E. faecalis*

To be filled out by the laboratory

Instructions: Please send data of the first **blood and/or cerebrospinal fluid (CSF)** isolate of all patients with an invasive *E. faecium* or *E. faecalis* infection (please specify by ticking relevant box above). Use 1 form per isolate.

Laboratory Code		
Isolate ID	Isolate source ☐ Blood ☐ CSF	Date of sample collection (yyyy-mm-dd)
Patient ID	Gender ☐ Male ☐ Female ☐ Other ☐ Unknown	Year of birth (yyyy)
Hospital ID	Origin of patient ☐ Inpatient ☐ Outpatient ☐ Other ☐ Unknown	Date of admission (yyyy-mm-dd)

Hospital Department

☐ Internal medicine ☐ Paediatrics/neonatal ☐ Paediatrics/neonatal ICU ☐ Surgery ☐ Haematology/Oncology
☐ Obstetrics/Gynaecology ☐ Intensive care unit (ICU) ☐ Emergency department ☐ Urology ☐ Infectious disease
☐ Other ☐ Unknown

Antibiotic susceptibility testing (S/I/R, zone **and/or** MIC) MIC can be done by broth dilution or gradient strip test

Antibiotic	SIR (final interpretation result of all different susceptibility test performed) Fill in S, I or R	Zone diameter (mm)	Zone diameter interpretation Fill in S, I or R	MIC Automated system (mg/L)	MIC interpretation Automated system Fill in S, I or R	MIC Gradient strip test/ agar dilution/ microdilution (mg/L)	MIC interpretation Gradient strip test/agar dilution/micro-dilution Fill in S, I or R
Ampicillin or Amoxicillin	☐ ☐	☐☐ –	☐ –	- - - - - - - - - - - -	☐ ☐	- - - - - - - - - - - -	☐ ☐
High-level gentamicin*	☐	☐☐	☐	- - - - - -	☐	- - - - - -	☐
Vancomycin	☐	☐☐	☐	- - - - - -	☐	- - - - - -	☐
Linezolid	☐	☐☐	☐	- - - - - -	☐	- - - - - -	☐

* High-level gentamicin is tested to screen for high-level aminoglycoside resistance (HLAR). If screen for HLAR is negative, report result as "S"; if screen for HLAR is positive, report result as "R".

Send this form to (Name/Institute/Contact details):

ANNEX

D

Manual electronic isolate record form

Contents

1. Introduction

The electronic isolate record form (eIRF) is an Excel workbook designed to enter and edit CAESAR data. The eIRF contains a data entry form that has the same structure as the paper-based isolate record forms (IRFs). This allows for electronic capture of CAESAR data at local laboratory level without the need for a dedicated laboratory information system. The data are stored in an Excel spreadsheet in the required CAESAR data format. The eIRF can be translated into any language and configured to fit the local situation. Some languages are already configured, and can be selected from the "Start" sheet. Translations into other languages are available on request.

This annex describes how to use and configure the eIRF. Section 2 describes the eIRF and its contents. Instructions on how to use the eIRF are in section 3. Section 4 provides detailed instructions on configuring the eIRF. Section 5 describes how to create isolate level data sheets to facilitate analysis. Finally, section 6 provides information on language versions of the eIRF.

Contact the CAESAR data manager by email (caesar@rivm.nl) to obtain the most recent version of the eIRF, to get help configuring the eIRF or to get another language version.

2. The eIRF

2.1 Opening the eIRF

The eIRF is a multisheet, macro-enabled Excel workbook, which can be opened from within Excel or Windows explorer. The eIRF will only work if macros are enabled, by clicking "Enable Content" (Fig. D.1).

Fig. D.1. Opening the eIRF and enabling macros
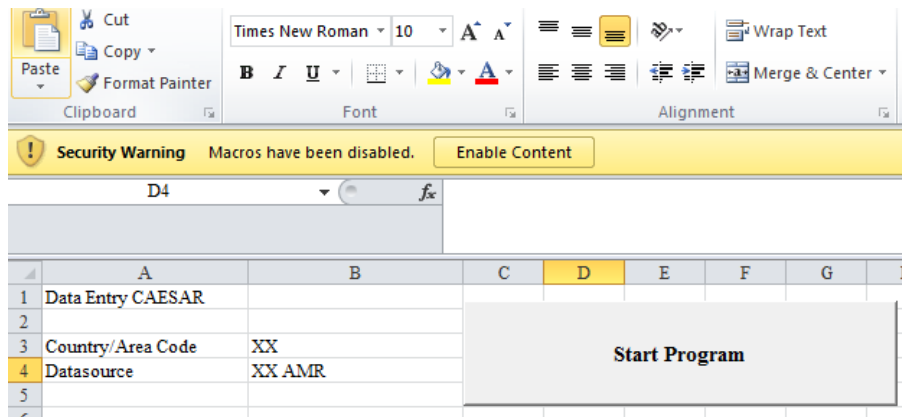
However, sometimes the option to enable the content is not available. This is because the antivirus protection settings prohibit macro execution in files originating from external sources (e.g. files downloaded from email or internet) (Fig D.2).

Fig. D.2. Macros blocked
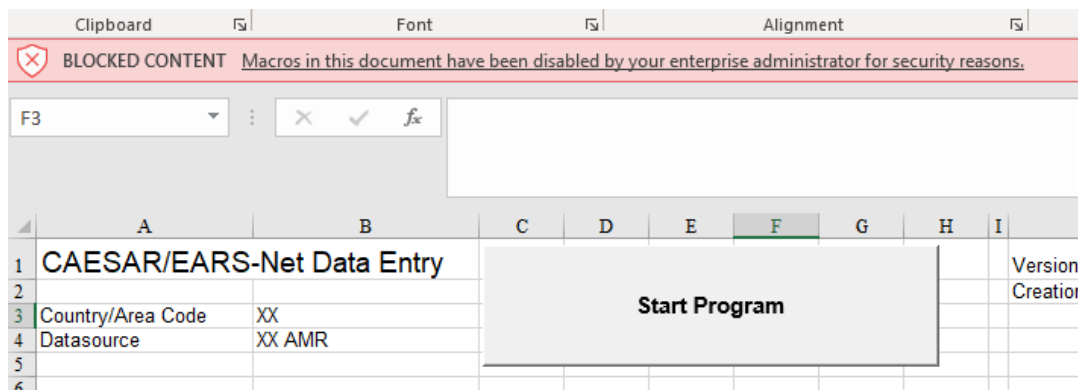
This can be solved by marking the file as safe, which can be done using Windows Explorer. First ensure that the file is closed, then click on the file in Windows Explorer using the right mouse button and choose "Properties". A form will open (Fig. D.3). In the form, check the "Unblock" box and then click "OK". Open the file and click on "Enable Content".

Fig. D.3 eIRF File properties
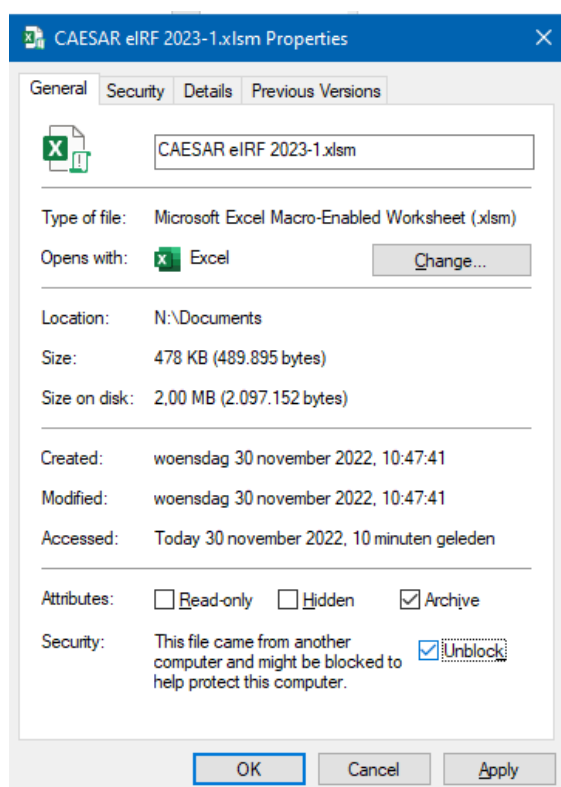
As an Excel file, the eIRF can be edited, saved and copied. Before making changes or entering data in the eIRF, use "Save as" to save a copy of the original file and give it a descriptive name. That way, it is possible to go back to the original file if mistakes are made during editing or to enter a new set of data.

2.2 Sheets in the eIRF

The eIRF contains several sheets (Table D.1). The data entry form can be opened from the "Start" sheet; data are stored and can be viewed in the "Data" sheet. The other sheets are used to translate and configure the form.

Table D.1. eIRF sheets

Sheet	Description
Start	This sheet allows access to the data entry form ("Data" sheet). Click the "Start Program" button to open the eIRF main screen. To use the eIRF in another language, click on the desired language button before clicking the "Start Program" button.
Data	This sheet stores the data in the CAESAR format. The header is already there; even if the sheet is empty.
Pathogens	This sheet contains the pathogens under CAESAR surveillance using The European Surveillance System (TESSy) codes; these are the same pathogens listed in the data entry form. Column B ("English Name") and column C ("Local names") list the standard (Latin) names of the pathogens, which are used in most languages. To use non-English pathogen names, edit column C to replace the English names with translated ones. The names in column C are visible in the data entry form. This sheet may also be used to add additional pathogens included in national surveillance (see section 4.3).

Table D.1. Contd.

Sheet	Description
Antibiotics	This sheet contains the WHONET codes and the names of all antibiotics listed in the data entry form. English terms are listed in column B ("English Name"), whereas translated terms can be entered in column C ("Local name") to override the default English antibiotic names. Again, the names in column C are displayed in the data entry form. Use this sheet to add antibiotics used in local laboratories by adding a row with the code and the name of the antibiotic (see section 4.4).
Isolate Source XXX (where XXX is the language code)	This sheet contains the specimen types from which pathogens can be isolated and the corresponding WHONET codes. Only the sheet for the selected language is visible. English terms are listed in column B, whereas translated terms can be entered in column C to override the default English terms. It already contains CAESAR specimens (i.e. blood and cerebrospinal fluid), but other specimen types can be added in a separate row. WHONET codes for additional specimen types are available (send requests to caesar@rivm.nl).
PathogenAntibiotic	This sheet contains all pathogen–antibiotic combinations listed in the data entry form, using codes for pathogens and antibiotics. Pathogen–antibiotic combinations can be added using the same codes as in the "Pathogen" and "Antibiotics" sheets. Column C ("Disk"), column E ("AUTO") and column F ("Gradient") contain a 1 or a 0. These are used to configure the data entry form to indicate which results can be entered. More information on configuration is available in section 4.5. Column D ("Disk Load") contains the disk load of the disks used for this combination. Columns G to J contain the EUCAST antibiotics breakpoints. Column K can be used to define the order of the antibiotics in the entry form. If you make changes, these will take effect after clicking "Start Program" again.
Pathogen–Resistance Mechanism	This sheet lists all combinations of pathogens and phenotypic or genotypic tests for specific mechanisms of antimicrobial resistance used in the data entry form. If a specific resistance mechanism test is relevant for a certain pathogen, the data entry form has a field to enter the result. For example, a polymerase chain reaction test for the presence of the MecA gene (coded MECA) is relevant for *S. aureus*, but not for other pathogens. In addition to the resistance mechanism tests, this sheet contains a field to enter the serotype for *Streptococcus pneumoniae* (coded SERO).
PatID lookup	The main screen has an option to encrypt all patient identifiers (IDs) by transforming patient IDs to numbers that hide patient information (see section 3.2). The encryption algorithm ensures that the same patient ID is always transformed to the same number. This process cannot be reversed. As a reference, this sheet creates a table with the original patient IDs and the encrypted patient IDs. To keep the original patient IDs confidential, delete (or empty) the PatID lookup sheet (after making a copy for reference) before sharing the data with others (e.g. the national AMR surveillance team, CAESAR).
Translation terms XXX (where XXX is the language code)	This sheet contains all texts used in the eIRF. Only the translation sheet for the selected language is visible. Column B ("English") should not be adapted, as it is meant for reference, so that people not familiar with the local language can understand the terms. The text in column C ("Local") is used in the eIRF. The terms in column C can be replaced by terms in any language; these translated terms will be shown in the eIRF. The procedures for translation are described in section 6. Selecting another language will make the translation sheet for the corresponding language visible.

3. Using the eIRF

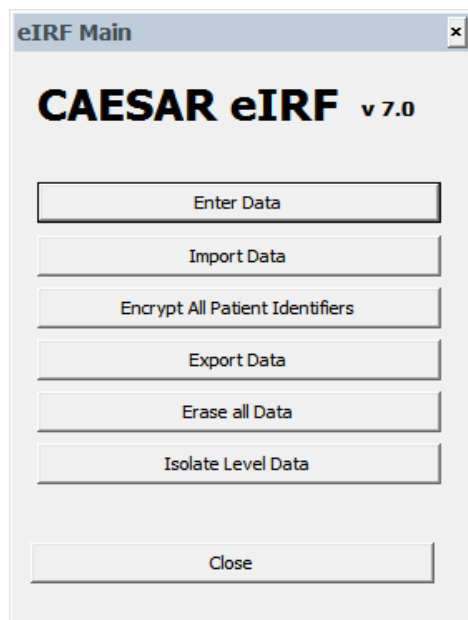## 3.1 Choosing the language and starting the program

On the "Start" sheet, choose the language to be used in the form, by clicking one of the language buttons (Fig. D.4). After selecting the language, start the program.

Fig. D.4. Selecting a language and starting the program on the "Start" sheet

Click on "Start Program" to open the main screen (Fig. D.5).

Fig. D.5. The eIRF main screen

3.2 The main eIRF screen

The main eIRF screen consists of seven buttons (Table D.2).

Table D.2. The main eIRF screen buttons

Button	Description
Enter Data	This button opens the data entry form ("Data" sheet), which enables manual data entry of susceptibility of single isolates. Section 3.3 describes the functionality of this form.
Import Data	This button opens a file selector to import a CAESAR CSV file (delimited by commas or semicolons). If the "Data" sheet already contains data, the imported data are added to the data in the sheet.
Encrypt All Patient Identifiers	This button is used to encrypt the patient identifiers (patient IDs) in the "Data" sheet by transforming the patient IDs to numbers that hide patient information. This is done by a one-way encryption. This means that reconstructing the patient ID from the generated number is impossible. To be able to find the original patient ID, the "PatID lookup" sheet automatically creates a table with the original patient IDs and the encrypted patient IDs. If this option is used, it is important to erase the "PatID lookup" sheet in the copy of the eIRF before sharing it with others (e.g. the national AMR surveillance team, CAESAR). Alternatively, share only the data, not the complete eIRF.
Export Data	This button exports all data in the "Data" sheet to a CAESAR CSV file. Choose the name and location of the exported file.
Erase all Data	After confirmation, this button deletes all contents of the "Data" sheet, leaving only the header row. This option is useful when making a new file for another data collection period, after saving the previous period. Be careful with this option, as it is not possible to restore erased data.
Isolate Level Data	This button converts data in the "Data" sheet to a format more convenient for analysis: one row per isolate instead of one row per antibiotic result. These data are added to the eIRF in additional sheets, one for each pathogen. More information on creating isolate level data sheets are in section 6.
Close	This button closes the eIRF main screen.

3.3 Manual data entry

3.3.1. The data entry form

The data entry form enables entry of the results of antimicrobial susceptibility tests. It is configured according to the data requirements in this publication, but can be adapted to suit the needs of a laboratory. See section 4 for more information on how to adapt the form.

Upon opening the form, the main screen will be displayed on the screen (Fig. D.5). From here, the Data Entry Screen can be accessed by pressing "Enter Data" (Fig. D.6).

Fig. D.6. The empty eIRF data entry form

The data entry form has fields to enter laboratory, hospital, patient and sample data. After selecting an organism from the species drop-down menu, antibiotic susceptibility data can be entered for an isolate (Fig. D.7).

Fig. D.7. The eIRF data entry screen with a pathogen selected

ANNEX D

Susceptibility test results (the crude test value as well as the SIR interpretation) can be entered for tests from disk diffusion, automated systems and gradient/dilution. When an MIC or zone value is entered, the SIR interpretation will be determined using the breakpoints in the "PathogenAntibiotic" sheet. The final SIR interpretation for each antibiotic is filled automatically when one of the SIR interpretations is present (Fig. D.8). When more than one interpretation is present, the gradient/dilution has preference over disk diffusion, and disk diffusion has preference over automated MIC. This can be overruled by manually selecting the final SIR interpretation.

Fig. D.8. The eIRF data entry form with data entered

Enter Data

CAESAR eIRF v 7.0　　　　　　　　　　　　　　　　　　　2019-03-07

Field	Value	Field	Value
Country/Area Code	XX	Patient ID	6326223911
Data Source	AMR surveillance	Gender	Female
Laboratory Code	XX001	Age	34
Guideline	EUCAST	Hospital Department	Surgery
Hospital ID	3	Type of Patient	Inpatient
Isolate ID	74926492	Date of Admission	2018-03-01
Date of Sample	2018-03-04		

Isolate Source: Blood

Species: Escherichia coli

Buttons: Back | Next Isolate | Delete Isolate | Print Form | Done

ESBL:　carbapenemase:

Antibiotic	Disk Load	Zone (mm)	Disk SIR	MIC (Automated)	Automated SIR	MIC (Gradient/Dilution)	Gradient SIR	Final SIR
Ampicillin	10	6	R	>=32	R			R
Amoxicillin	----		---					
Amoxicillin/Clavulanic acid	20/10	6	R			16	R	R
Piperacillin/Tazobactam	30/6	22	S					S
Cefotaxime	5	6	R					R
Ceftriaxone	30			>=64	R			R
Ceftazidime	10	15	R					R
Gentamicin	10	6	R	>=16	R			R
Tobramycin	10							
Amikacin	30	21	S					S
Ciprofloxacin	5			1	S			S
Ofloxacin	5							
Levofloxacin	5			<=0.12	S			S
Imipenem	10	30	S	<=0.25	S			S
Meropenem	10	31	S					S
Ertapenem	10			<=0.5	S			S
Colistin	----		---	----	---	----	---	

3.3.2 Data entry form buttons

After entering data, click one of the buttons (Table D.3).

Table D.3. Data entry form buttons

Button	Description
Back	This button does not save the data currently entered in the data entry form. It loads the previous isolate from the "Data" sheet into the data entry form.
Next Isolate	This button stores data from the data entry form in the "Data" sheet, one row for each antibiotic tested. Then it clears fields in the form, except those on country/area, laboratory and hospital. If one or more mandatory fields are left blank, an alert will open, listing the required data that need to be added. Note: it is not possible to continue ("Next Isolate" or "Done") if mandatory data are missing.
Delete Isolate	If data on a new isolate were entered, clicking this button clears the form without saving the data. If an existing isolate was loaded from the "Data" sheet, through the "Back" button, or with the edit functionality (described in section 3.4), the data from this isolate are removed from the "Data" sheet.
Print Form	This button sends the data entry form to a printer (if installed), which can be useful when a hard copy of the form is needed.
Done	This button stores data from the data entry form in the "Data" sheet and closes the form.

Clicking the "x" on the upper right corner of the form closes the form without saving data.

3.4 Editing previously entered data

All data are stored in the "Data" sheet with results for each antibiotic in a separate row (in the CAESAR format). To edit data on an isolate, select one of the rows of isolate data and right-click the mouse (this will open the Windows context menu, Fig. D.9). Select "Edit" on the bottom of the drop-down menu to open the data entry form, filled with data on the isolate. Edit the data and click "Done" or "Next Isolate" to save the changes. This will delete all original information on the isolate and replace it with the new data entered (also for the antibiotics that were not corrected) on the bottom of the "Data" sheet. To edit the last entry, or the isolate on the bottom of the "Data" sheet, click "Back" on the data entry form. The form will be filled with data from the last isolate.

Fig. D.9. Windows context menu showing how to edit previously entered data

3.5 Saving the entered data

After entering or editing data, save the eIRF. If the data are complete, choose "Export Data" on the main screen to create a dataset in the CAESAR format. The resulting CSV file can be sent to CAESAR.

4. Configuring the eIRF

4.1 Purpose

Configuring the eIRF allows the user to decide which pathogens, antibiotics and resistance mechanism tests to display. Use the sheets within the eIRF to configure it. Programming skills are not necessary.

4.2 Country/Area code and data source

Every record must have a country/area code (the two-character code of the country/area) and a data source. This information is the same for each record, so enter it once in the "Start" sheet (Fig. D.10). The country/area code is the two characters used as an Internet suffix (e.g. "NL" for the Netherlands). The data source is the surveillance system from which the data originates. For surveillance systems that do not have a name, use the name <country code>-AMR.

Fig. D.10. Country/Area code and data source on the "Start" sheet

4.3 Editing the list of pathogens

The "Pathogens" sheet contains the nine pathogens under CAESAR surveillance and their corresponding TESSy pathogen codes (Fig. D.11). The pathogens in this sheet populate the "Species" drop-down menu in the data entry form (Fig. D.12).

Fig. D.11. Pathogens under CAESAR surveillance in the "Pathogens" sheet

	A	B	C
1	Code	English Name	Local name
2	strpne	Streptococcus pneumoniae	Streptococcus pneumoniae
3	staaur	Staphylococcus aureus	Staphylococcus aureus
4	encfae	Enterococcus faecalis	Enterococcus faecalis
5	encfai	Enterococcus faecium	Enterococcus faecium
6	esccol	Escherichia coli	Escherichia coli
7	klepne	Klebsiella pneumoniae	Klebsiella pneumoniae
8	pseaer	Pseudomonas aeruginosa	Pseudomonas aeruginosa
9	acispp	Acinetobacter species	Acinetobacter species
10	salspp	Salmonella species	Salmonella species

In the data entry form, use a drop-down menu to select the pathogen (Fig. D.12).

Fig. D.12. Selecting a pathogen in the data entry form

Use this sheet to add a new row for each additional pathogen included in national surveillance and the corresponding TESSy code. As an example, Fig. D.13 shows the "Pathogens" sheet after adding the pathogen *Klebsiella oxytoca*.

Fig. D.13. *Klebsiella oxytoca* added to the "Pathogens" sheet

	A	B	C
1	Code	English Name	Local name
2	strpne	Streptococcus pneumoniae	Streptococcus pneumoniae
3	staaur	Staphylococcus aureus	Staphylococcus aureus
4	encfae	Enterococcus faecalis	Enterococcus faecalis
5	encfai	Enterococcus faecium	Enterococcus faecium
6	esccol	Escherichia coli	Escherichia coli
7	klepne	Klebsiella pneumoniae	Klebsiella pneumoniae
8	pseaer	Pseudomonas aeruginosa	Pseudomonas aeruginosa
9	acispp	Acinetobacter species	Acinetobacter species
10	salspp	Salmonella species	Salmonella species
11	kleoxy	Klebsiella oxytoca	Klebsiella oxytoca

The drop-down menu in the data entry form shows that the pathogen was added (Fig. D.14).

Fig. D.14. Selecting a pathogen in the data entry form after adding *Klebsiella oxytoca*

Species

- Streptococcus pneumoniae
- Staphylococcus aureus
- Enterococcus faecalis
- Enterococcus faecium
- Escherichia coli
- Klebsiella pneumoniae
- Pseudomonas aeruginosa
- Acinetobacter species
- Salmonella species
- Klebsiella oxytoca
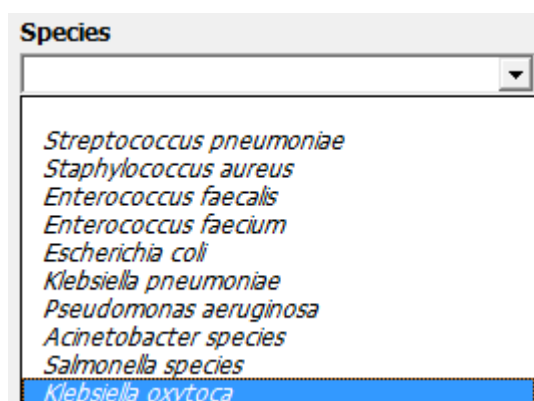
4.4 Editing the list of antibiotics

The "Antibiotics" sheet contains all the antibiotics from WHONET. Use this sheet to add a new row for each additional antibiotic used by laboratories (and not already listed). Enter a code and the name of the antibiotic in both the "English Name" and "Local name" columns. Contact the CAESAR data manager by email (caesar@rivm.nl) for codes not included in WHONET.

4.5 Editing the list of pathogen–antibiotic combinations

For each pathogen under CAESAR surveillance, the antibiotics included in the minimum panel to report to CAESAR are listed in the "PathogenAntibiotic" sheet (Fig. D.15). Column C ("Disk"), column E ("AUTO") and column F ("Gradient") contain either:

- "1" if data for this type of test and pathogen–antibiotic combination can be entered in the data entry form; or

- "0" (zero) if data for this type of test and pathogen–antibiotic combination cannot be entered in the data entry form.

For example, as vancomycin (VAN) disk diffusion results are not reliable for *S. aureus*, a value of "0" in the "Disk" column ensures that disk diffusion results cannot be entered in the data entry form. Similarly, the norfloxacin (NOR) disk is used as a screening for fluoroquinolone susceptibility in *S. aureus*. Measuring an MIC is not applicable for norfloxacin and therefore entering MIC results is disabled in the data entry form (configured by "0" in the columns "AUTO" and "Gradient" (Fig. D.15)).

Fig. D.15. The "PathogenAntibiotic" sheet

	A	B	C	D	E	F
1	Pathogen ▼	Antibiotic	Disk	Disk Load	AUTO	Gradient
2	strpne	PEN	1	23	1	1
3	strpne	OXA	1	1	0	0
4	strpne	ERY	1	15	1	1
5	strpne	CLR	0		1	1
6	strpne	AZM	0		1	1
7	strpne	CTX	0		1	1
8	strpne	CRO	0		1	1
9	strpne	NOR	1	10	0	0
10	strpne	LVX	1	5	1	1
11	strpne	MFX	1	5	1	1
12	staaur	FOX	1	30	1	1
13	staaur	OXA	0		1	1
14	staaur	CIP	1	5	1	1
15	staaur	LVX	1	5	1	1
16	staaur	OFX	1	5	1	1
17	staaur	NOR	1	10	0	0

To see the antibiotics for one species, use the drop-down menu (arrow) in the "Pathogen" header field (column A) to select the species (using the Excel filter function, Fig. D.16). After selecting *S. aureus* for example, the sheet only displays pathogen–antibiotic combinations for *S. aureus* (Fig. D.17).

Fig. D.16. Using a filter to select rows for *S. aureus*
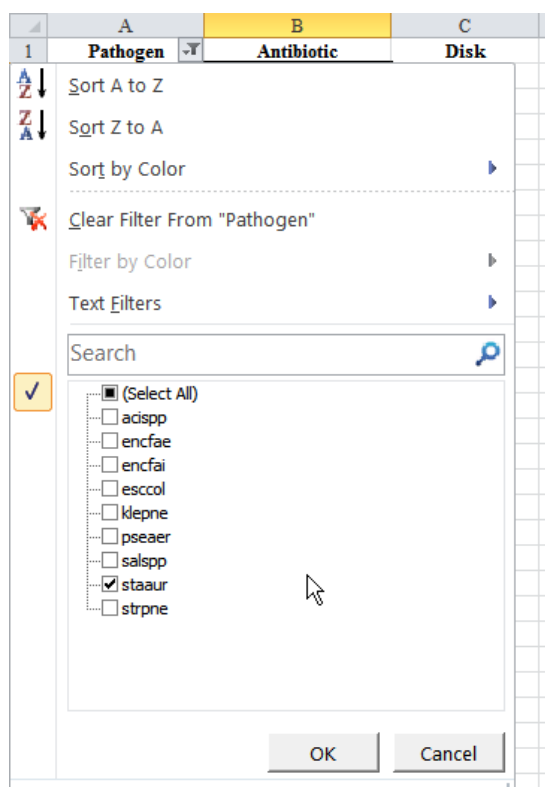
Fig. D.17. The "PathogenAntibiotic" sheet with _Staphylococcus aureus_ (staaur) selected

	A	B	C	D	E	F
1	**Pathogen**	**Antibiotic**	**Disk**	**Disk Load**	**AUTO**	**Gradient**
12	staaur	FOX	1	30	1	1
13	staaur	OXA	0		1	1
14	staaur	CIP	1	5	1	1
15	staaur	LVX	1	5	1	1
16	staaur	OFX	1	5	1	1
17	staaur	NOR	1	10	0	0
18	staaur	RIF	1	5	1	1
19	staaur	LNZ	1	10	1	1
20	staaur	VAN	0		1	1
91						

Use this sheet to add or modify pathogen–antibiotic combinations to fit with local practice.

For example, the CAESAR manual recommends using cefoxitin (FOX) disk diffusion for MRSA detection; alternatively an oxacillin (OXA) MIC method could be used, but doing both tests is not useful. It is recommended to delete either FOX or OXA from the sheet if one of these antibiotics is not tested in a given country/area or laboratory, for example, a laboratory that uses cefoxitin disk diffusion and does not test oxacillin. The configuration for this example is in Fig. D.18 (filtered for "staaur").

The "Disk Load" column gives the disk potency used. The initial values in this column are according to EUCAST AST guidelines but should be adjusted if other disk potencies are used.

Fig. D.18. The "PathogenAntibiotic" sheet with Staphylococcus _aureus_ ("staaur") configured to suit local procedures

A	B	C	D	E	F
Pathogen	**Antibiotic**	**Disk**	**Disk Load**	**AUTO**	**Gradient**
staaur	FOX	1	30	1	1
staaur	CIP	1	5	1	1
staaur	LVX	1	5	1	1
staaur	OFX	1	5	1	1
staaur	NOR	1	10	0	0
staaur	RIF	1	5	1	1
staaur	LNZ	1	10	1	1
staaur	VAN	0		1	1

Note: completely delete unused rows and not just the cell contents. Empty rows will disrupt the program.

This information configured in the "PathogenAntibiotic" sheet is displayed in the data entry form (Fig. D.19).

Fig. D.19. The data entry form for Staphylococcus *aureus* according to local configuration; entry of certain test results disabled

4.6 Selecting resistance mechanisms tests

The "Pathogen–Resistance Mechanism" sheet lists all combinations of pathogens and phenotypic and genotypic tests for specific mechanisms of antimicrobial resistance used in the data entry form (Fig. D.20). Use this sheet to change the resistance mechanism tests displayed in the data entry form.

Fig. D.20. Data in the "Pathogen–Resistance Mechanism" sheet

A	B
Pathogen	**Confirmation**
esccol	ESBL
esccol	CARB
klepne	ESBL
klepne	CARB
staaur	PBP
staaur	MECA
strpne	SERO

This sheet lists two possible resistance mechanism tests for *S. aureus*: MECA (referring to a polymerase chain reaction test for the presence of the MecA gene) and penicillin binding protein (PBP) (PBP2a agglutination, an immunological test to confirm MRSA). It is unlikely that both tests are used, so one of these rows can be deleted.

4.7 Saving the configured eIRF

Save the eIRF after configuring it to follow local procedures. Use a descriptive filename, such as "eIRF <country>.xlsm" or "eIRF for <laboratory>.xlsm". The eIRF is now ready for data entry.

5. Creating isolate level data sheets

In the "Data" sheet, data are stored in a format with one antibiotic per row. Data from one isolate are stored in several rows. This format is specifically designed for data transfer to the CAESAR data manager. However, to perform local or national data analyses, it is often more convenient to have a format with a single row for each isolate and separate columns for each antibiotic. For instance, this data format can be used to see resistance patterns and to aggregate the results in antibiotic classes.

The button "Isolate Level Data" on the eIRF main screen creates data sheets in which data from the "Data" sheet are converted into a format of one row per isolate. Because each species has its own antibiotic panel, the eIRF will have a separate sheet for each species (Fig. D.21).

Fig. D.21. Isolate level data sheets, one for each species

It is possible to create sheets for one, several or all species in one step (Fig. D.22). This step can take several minutes, especially when selecting all species, depending on the size of the data file. This functionality is limited to the pathogens under CAESAR surveillance and the antibiotics listed in the minimum panel for reporting in the CAESAR manual. Fig. D.23 shows an example of an isolate level data sheet.

Fig. D.22. Selecting pathogens for isolate level data conversion

The isolate level data sheet gives the results for all the antibiotics in the minimum panel for CAESAR reporting, and a result for the antibiotic groups or classes as reported by CAESAR, based on the final SIR interpretation. The rules for group results are:

- if one antibiotic in a group is R, then the group is R

- if one antibiotic in a group is I, and none is R, then the group is I

- if all antibiotics in a group are S, then the group is S

- if none of the antibiotics in the group is tested, then the group result is empty.

The names of the groups are the code of the antibiotics in that group, separated by underscores (Fig. D.23). For example, the name of the group consisting of ciprofloxacin, levofloxacin and ofloxacin is "CIP_LVX_OFX". This name is preferred over "Fluoroquinolones", because it lists the antibiotics in the group.

Fig. D.23. Isolate level data sheet for *E. coli*, with group results for antibiotic classes

I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W
IsolateID	HospitalII	PatientTy	HospitalU	DateofHo	ESBL	ResultCarbape	Guideline	CIP_LVX_OFX	GEN_TOB	CTX_CRO	IPM_MEM	AMC	AMC_DIAM	AMC_DI
16999	001X	INPAT	ICU		NEG	UNK	EUCAST	R	R	R	R	R	6	20/10ug
27414	001X	INPAT	PEDS		POS	UNK	EUCAST	R	R	R	S	R	15	20/10ug
41571	001X	INPAT	INTMED		POS	UNK	EUCAST	R	R	R	S	R	14	20/10ug
56382	001X	INPAT	INFECT		NEG	UNK	EUCAST	S		S	S	S	24	20/10ug
85783	001X	INPAT	PEDS		POS	UNK	EUCAST	S	R	R	S	R	18	20/10ug
94062	001X	INPAT	PEDS		POS	UNK	EUCAST	S	R	R	S	R	6	20/10ug
104067	001X	INPAT	PEDS		POS	UNK	EUCAST	R	R	R	S	R	15	20/10ug
106191	001X	INPAT	ICU		NEG	UNK	EUCAST	R	R	R	R	R	6	20/10ug
152715	001X	INPAT	INTMED		NEG	UNK	EUCAST	R	S	R	S	R	15	20/10ug
153779	001X	INPAT	PEDS		NEG	UNK	EUCAST	S		S	S	S	26	20/10ug
157780	001X	INPAT	PEDS		NEG	UNK	EUCAST	I		S	S	S	20	20/10ug
157786	001X	INPAT	PEDS		NEG	UNK	EUCAST	R	R	R	S	R	6	20/10ug
160218	001X	INPAT	INTMED		NEG	UNK	EUCAST	R	R	R	R	R	6	20/10ug
161739	001X	INPAT	INFECT		NEG	UNK	EUCAST	R	R	R	R	R	6	20/10ug
168217	001X	INPAT	ED		NEG	UNK	EUCAST	R	R	R	R	R	6	20/10ug
170439	001X	INPAT	SURG		NEG	UNK	EUCAST	R	R	R	R	R	6	20/10ug
170443	001X	INPAT	SURG		NEG	UNK	EUCAST	R	R	R	R	R	6	20/10ug

6. Language versions of the eIRF

6.1 Requesting a new language version

The eIRF can be made available in any language upon request to the CAESAR data manager (caesar@rivm.nl). To be able to add the language to the eIRF, the CAESAR data manager will need the terms in the sheet "Translation terms XXX" in the requested language. With these translations, the CAESAR data manager can add the language to those available in the eIRF.

6.2 Editing an existing language version

Sometimes an English – instead of the translated – term may be in a form that is not English. This means that the translation for that term was not available when the language was incorporated. Find the English term is in the "Local names" column in the "Translation Terms XXX" sheet (where XXX is the language code). Replace the English term in the "Local names" column with the translated term, save and restart the eIRF to see the translated term. Similarly, if a translation is not correct, or another translation is preferred, the user can correct the term in the "Translation Terms XXX". When making corrections, please send the corrected eIRF to the CAESAR data manager, so that updated versions of the eIRF will contain the latest translations.

ANNEX

E

CAESAR variables for AMR surveillance

The set of variables for isolate-based AMR reporting consists of eight technical variables and 29 epidemiological variables, which are classified into variable levels: patient, sample, isolate, and AMR test level (Fig. E.1).

Fig. E.1. CAESAR variables
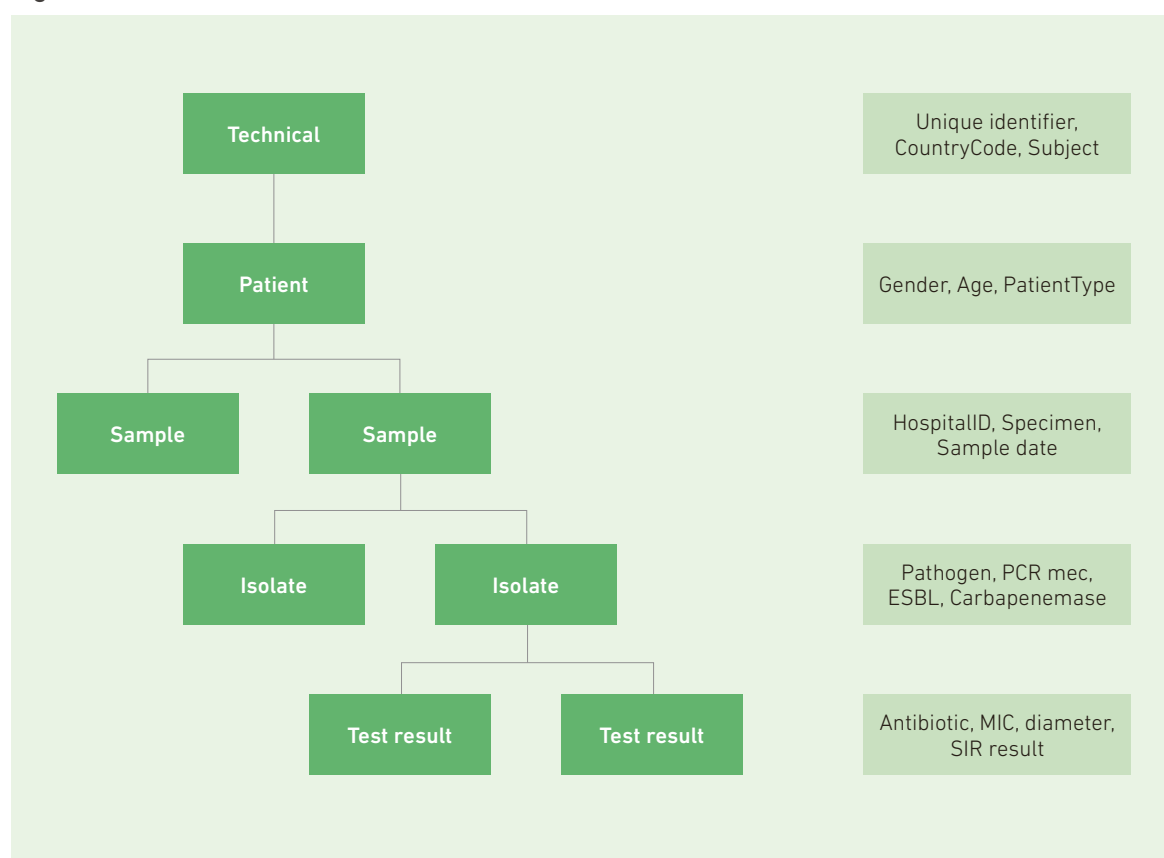
An overview of the variables is in Table E.1. The variables are described in more detail, including the validation rules, in Table E.2.

Table E.1. Overview of set of variables for isolate-based AMR reporting to CAESAR

Technical variables		
Variable name	**Mandatory**	**Explanation**
1. RecordID	Yes	Unique identifier (primary key)
2. RecordType	Yes	Fixed: AMRTEST
3. RecordTypeVersion	No	Version number of the variable set (now 2)
4. Subject	Yes	Fixed: AMR
5. DataSource	Yes	Code for local surveillance system
6. ReportingCountry	Yes	Country code
7. Status	No	NEW/UPDATE or DELETE
8. LaboratoryCode	Yes	Laboratory code: two letter country code + three digits laboratory no.
Epidemiological variables at patient level		
9. PatientCounter	Yes	Anonymized patient ID, must be numeric
10. Gender	No	Gender of the patient M/F/O/UNK
11. Age	No	Age of the patient in years
12. PatientType	No	Origin of patient: INPAT/OUTPAT/O/UNK
Epidemiological variables at sample level		
13. DateUsedForStatistics	Yes	Date of sample collection
14. Specimen	Yes	Material: BLOOD or CSF
15. IsolateId	No	Isolate sample identifier
16. HospitalId	No	Hospital code, recommended format laboratory no. + one letter
17. HospitalUnitType	No	Hospital department (see Table E.2)
18. DateOfHospitalisation	No	Date of admission, YYYY-MM-DD
Epidemiological variables at isolate level		
19. Pathogen	Yes	Pathogen code (see Table E.2)
20. ResultPCRmec	No	PCR mec-gene: POS/NEG/UNK
21. ResultPbp2aAggl	No	PBP2a-agglutination: POS/NEG/UNK
22. Serotype	No	Serotype coded according to the Danish system[a]
23. ESBL	No	ESBL present: POS/NEG/UNK

Table E.1. Contd.

24. ResultCarbapenemases	No	Carbapenase detected: POS/NEG/UNK
Epidemiological variables at AMR test level		
25. Antibiotic	Yes	Antibiotic code (see Table E.2)
26. SIR	Yes	Interpretation of susceptibility: S/I/R
27. ResultZoneSign	No	Sign used in the zone diameter (> < =)
28. ResultZoneValue	No	Zone value (mm)
29. ResultZoneSIR	No	Interpretation of susceptibility from the zone: S/I/R
30. ResultMICSign	No	Sign used in the MIC (> < =)
31. ResultMICValue	No	MIC (mg/L)
32. ResultMICSIR	No	Interpretation of susceptibility from the MIC: S/I/R
33. ResultEtestSign	No	Sign used in the MIC from a gradient strip test (> < =)
34. ResultEtestValue	No	MIC value from gradient strip test (mg/L)
35. ResultEtestSIR	No	Interpretation of susceptibility from the gradient strip test: S/I/R
36. DiskLoad	No	Disk load (text)
37. ReferenceGuidelinesSIR	No	The guideline used (CLSI/EUCAST/NAT/O)

a   Geno KA, Gilbert GL, Song JY, Skovsted IC, Klugman KP, Jones C, et al. Pneumococcal capsules and their types: past, present, and future. Clin Microbiol Rev. 2015;(3):871–99. doi:10.1128/CMR.00024-15.

Variables are described in more detail in Table E.2. Mandatory variables are essential for the correct interpretation of the dataset. If a mandatory variable is missing, the record will be ignored in analysis.

Required variables are important variables and essential for correct analysis of the data. If a required variable contains missing data, the record will still be included for analysis.

Table E.2. Complete view; set of variables for isolate-based AMR reporting to CAESAR

Technical variables	
VariableName	**RecordID**
Description	Unique anonymized identifier for each record within and across the national surveillance system and subject – Member State selected and generated. Recommended format: "[ReportingCountry][LaboratoryCode] [Patient Counter][Pathogen] [Specimen][Antibiotic][DateUsedForStatistics]"
Mandatory	Yes
Required	Yes
Data type	String (Max length: 80)

Table E.2. Contd.

VariableName	RecordType
Description	Structure and format of the data
Mandatory	Yes
Required	Yes
Data type	Coded Value
Code	AMRTEST (fixed value for CAESAR)

VariableName	RecordTypeVersion
Description	There may be more than one version of a RecordType. This element indicates which version the sender uses when generating the message.
Mandatory	No
Required	No
Data type	Numeric
Code	2 (CAESAR uses AMRTEST version 2)

VariableName	Subject
Description	Subject of the data to report
Mandatory	Yes
Required	Yes
Data type	Coded Value
Code	AMR (fixed value for CAESAR)

VariableName	DataSource
Description	The data source (surveillance system) that the record originates from
Mandatory	Yes
Required	Yes
Data type	Coded Value
Code	<Name of the national surveillance> to be decided, and then fixed

VariableName	ReportingCountry
Description	The country reporting the record
Mandatory	Yes
Required	Yes

Table E.2. Contd.

Data type	Coded Value
Code	Two letter country codes, same as Internet suffix
VariableName	**Status**
Description	Status of reporting NEW/UPDATE or DELETE Default if left out: NEW/UPDATE. If set to DELETE, the record with the given RecordID will be deleted from the database (or better stated, invalidated). If set to NEW/UPDATE or left empty, the record is entered into the database. If the same record was already present, that record will be replaced.
Mandatory	No
Required	No
Data type	Coded Value
Code	NEW/UPDATE OR DELETE
VariableName	**LaboratoryCode**
Description	Laboratory code unique for each laboratory within the country
Mandatory	Yes
Required	Yes
Data type	Coded Value Recommended format: [ReportingCountry]-[code of three characters]
Epidemiological variables at patient level	
VariableName	**PatientCounter**
Description	Numeric code for each patient, unique within the laboratory; anonymous code by laboratory to specify patient
Mandatory	Yes
Required	Yes
Data type	Numeric (integer)
Code	Required that laboratories anonymize the PatientCounter
VariableName	**Gender**
Description	Gender
Mandatory	No (but with warning)
Required	Yes
Data type	Coded Value
Code	M = Male; F = Female; O = Other; UNK = Unknown

Table E.2. Contd.

VariableName	Age
Description	Age of the patient in years when the sample was taken
Mandatory	No (but with warning)
Required	Yes
Data type	Numeric
Code	Integer

VariableName	PatientType
Description	Origin of patient: was the patient admitted in a hospital (inpatient) at the moment the isolate was taken or not (outpatient). Patients who go to the hospital for dialysis, other day hospital care and to the emergency room should be classified as O for the field "PatientType". All other patients who are admitted in the hospital as inpatients should be classified as INPAT.
Mandatory	No (but with warning)
Required	Yes
Data type	Coded Value
Code	INPAT = Admitted (Inpatient); OUTPAT = Outpatient; O = Other (e.g. emergency room); UNK = Unknown

Epidemiological variables at sample level	
VariableName	DateUsedForStatistics
Description	Date when sample was taken
Mandatory	Yes
Required	Yes
Data type	Date
Code	Exact date only, "YYYY-MM-DD"

VariableName	Specimen
Description	The source of the isolate (e.g. blood)
Mandatory	Yes
Required	Yes
Data type	Coded Value
Code	BLOOD = blood; CSF = cerebrospinal fluid

VariableName	IsolateId
Description	Isolate ID; code for each isolate, unique within laboratory and year; Text code assigned by laboratory to specify isolate

Mandatory	No (but with warning)
Required	Yes
Data type	Text
VariableName	**HospitalId**
Description	Unique identifier for the hospital within each laboratory
Mandatory	No (but with warning)
Required	Yes
Data type	Text
Code	Unique identifier for the hospital within each laboratory; recommended format: [LaboratoryCode]-[letter assigned to a hospital – starting from A, B, C, etc.]
VariableName	**HospitalUnitType**
Description	Hospital department (at sample collection)
Mandatory	No (but with warning)
Required	Yes
Data type	Coded Value
Code	INTMED = Internal Medicine; PEDS = Paediatrics/neonatal; PEDSICU = Paediatrics/neonatal ICU; SURG = Surgery; ONCOL = Haematology/Oncology; OBGYN = Obstetrics/Gynaecology; ICU = Intensive Care Unit; ED = Emergency Department; URO = Urology; INFECT = Infectious Disease; O = Other; UNK = Unknown
VariableName	**DateOfHospitalisation**
Description	Date of admission in hospital
Mandatory	No
Required	No
Data type	Date
Code	Exact date only, "YYYY-MM-DD"
Epidemiological variables at isolate level	
VariableName	**Pathogen**
Description	Pathogen species and genus of the pathogen that has been isolated from the sample
Mandatory	Yes
Required	Yes

Data type	Coded Value
Code	ESCCOL = *Escherichia coli* KLEPNE = *Klebsiella pneumoniae* PSEAER = *Pseudomonas aeruginosa* ACISPP = *Acinetobacter* spp. SALSPP = *Salmonella* spp. STAAUR = *Staphylococcus aureus* STRPNE = *Streptococcus pneumoniae* ENCFAE = *Enterococcus faecalis* ENCFAI = *Enterococcus faecium*
VariableName	**ResultPCRmec**
Description	Detection of PCR mecA-gene
Mandatory	No
Required	No
Data type	Coded Value
Code	POS = positive; NEG = negative; UNK = unknown
Validation rule	To be reported only if Pathogen = STAAUR
VariableName	**ResultPbp2aAggl**
Description	Detection of PBP2a-agglutination
Mandatory	No
Required	No
Data type	Coded Value
Code	POS = positive; NEG = negative; UNK = unknown
Validation rule	To be reported only if Pathogen = STAAUR
VariableName	**Serotype**
Description	Serotype/group of the pathogen isolated from the sample. Reference: Danish Kauffman-Lund scheme[a] from the former WHO Collaborating Centre for Reference and Research on Pneumococci at Statens Serum Institute in Denmark.
Mandatory	No
Required	No
Data type	Coded Value
Code	Serotype (number; e.g. 1, or number + letter; e.g. 6A)
Validation rule	To be reported only if Pathogen = STRPNE

[a] Geno KA, Gilbert GL, Song JY, Skovsted IC, Klugman KP, Jones C, et al. Pneumococcal capsules and their types: past, present, and future. Clin Microbiol Rev. 2015;(3):871–99. doi:10.1128/CMR.00024-15.

Table E.2. Contd.

VariableName	ESBL
Description	Detection of ESBL
Mandatory	No
Required	No
Data type	Coded Value
Code	POS = positive; NEG = negative; UNK = unknown
Validation rule	To be reported only if Pathogen = ESCCOL or KLEPNE
VariableName	**ResultCarbapenemases**
Description	Detection of carbapenemases; this refers to phenotypic test for carbapenemase activity (e.g. the modified Hodge test – MHT).
Mandatory	No
Required	No
Data type	Coded Value
Code	POS = positive; NEG = negative; UNK = unknown
Validation rule	To be reported only if Pathogen = ESCCOL or KLEPNE or PSEAER.

Epidemiological variables at AMR test level

VariableName	Antibiotic
Description	Antibiotic code
Mandatory	Yes
Required	Yes
Data type	Coded Value
Code	AMC: amoxicillin-clavulanic acid; AMK: amikacin; AMP: ampicillin; AMX: amoxicillin; AZM: azithromycin; CAZ: ceftazidime; CIP: ciprofloxacin; CLR: clarithromycin; COL: colistin; CRO: ceftriaxone; CTX: cefotaxime; ERY: erythromycin; ETP: ertapenem; FEP: cefepime; FOX: cefoxitin; GEH: high-level gentamicin; GEN: gentamicin; IPM: imipenem; LNZ: linezolid; LVX: levofloxacin; MEM: meropenem; MFX: moxifloxacin; MIC: minimum inhibitory concentration; OFX: ofloxacin; OXA: oxacillin; PEN: penicillin; RIF: rifampicin; TOB: tobramycin; TZP: piperacillin-tazobactam; VAN: vancomycin
Validation rule	If you enter data that does not meet the requested combination of "Specimen", "Pathogen" and "Antibiotic", the record is ignored. Ignored means that data on these combinations will not be reported.
VariableName	**SIR**
Description	Final interpretation result of all different susceptibility tests performed
Mandatory	Yes

Required	Yes
Data type	Coded Value
Code	S = Susceptible, standard dosing regimen; I = Susceptible, increased exposure; R = Resistant
VariableName	**ResultZoneSign**
Description	Zone (> < =); this field can indicate if a value of the zone diameter of the disk test is "less than" (<); "equal to or less than" (< =); "equal to" (=); "equal to or greater than" (>=); or "greater than" (>) the value indicated in the following field.
Mandatory	No
Required	No
Data type	Coded Value
Code	<; < =; =; = >; >
VariableName	**ResultZoneValue**
Description	Zone (Value in mm)
Mandatory	No
Required	No
Data type	Numeric
Code	Integer
VariableName	**ResultZoneSIR**
Description	Interpretation of the zone test
Mandatory	No
Required	No
Data type	Coded Value
Code	S = Susceptible, standard dosing regimen; I =Susceptible, increased exposure; R = Resistant
VariableName	**ResultMICSign**
Description	MIC (> < =); this field can indicate if a value of the zone diameter of the MIC test is "less than" (<); "equal to or less than" (< =); "equal to" (=); "equal to or greater than" (>=); or "greater than" (>) the value indicated in the following field.
Mandatory	No
Required	No
Data type	Coded Value
Code	<; < =; =; = >; >

Table E.2. Contd.

VariableName	ResultMICValue
Description	MIC (Value in mg/L)
Mandatory	No
Required	No
Data type	Numeric
VariableName	**ResultMICSIR**
Description	Interpretation of the MIC test
Mandatory	No
Required	No
Data type	Coded Value
Code	S = Susceptible, standard dosing regimen; I = Susceptible, increased exposure; R = Resistant
VariableName	**ResultEtestSign**
Description	Gradient strip test (> < =); this field can indicate if a value of the zone diameter of the gradient strip test is "less than" (<); "equal to or less than" (< =); "equal to" (=); "equal to or greater than" (>=); or "greater than" (>) the value indicated in the following field.
Mandatory	No
Required	No
Data type	Coded Value
Code	<; < =; =; = >; >
VariableName	**ResultEtestValue**
Description	Gradient strip test MIC (Value in mg/L)
Mandatory	No
Required	No
Data type	Numeric
Code	If <1 then float, if >=1 then integer; the value 1.5 is also allowed
VariableName	**ResultEtestSIR**
Description	Interpretation of the gradient strip test
Mandatory	No
Required	No

Table E.2. Contd.

Data type	Coded Value
Code	S = Susceptible, standard dosing regimen; I = Susceptible, increased exposure; R = Resistant
VariableName	**DiskLoad**
Description	Disk content (only if Zone); this field can be used to mention the load of the antibiotic disk used. Please mention the value and the units (e.g. mcg, Units or IU).
Mandatory	No
Required	No
Data type	Text
Code	Value and units: i.e. IU, mcg
VariableName	**ReferenceGuidelinesSIR**
Description	To differentiate use of CLSI and EUCAST guidelines for breakpoints
Mandatory	No
Required	No
Data type	Coded Value
Code	EUCAST = European Committee on Antimicrobial Susceptibility Testing CLSI = Clinical and Laboratory Standards Institute NAT = National O = Other

ANNEX

F

Pathogens under CAESAR surveillance

The following text on pathogens under CAESAR surveillance was adopted from the Antimicrobial resistance: global report on surveillance 2014 published by WHO *(1)* and the annual report of the EARS-Net published by the ECDC in 2015 *(2)*.

Escherichia coli

E. coli is part of the normal microbiota in the intestine in humans and animals. Nevertheless, it:

- is the most frequent cause of community-acquired and frequent cause of hospital-acquired urinary tract infections (including pyelonephritis);

- is the most frequent cause of bloodstream infection among people of all ages;

- is associated with intra-abdominal infections such as peritonitis;

- can cause meningitis in neonates; and

- is one of the leading causes of foodborne infections worldwide.

Infections with *E. coli* usually originate from the person affected (autoinfection), but strains with a particular resistance or disease-causing properties can also be transmitted from animals, through the food chain or between individuals.

Klebsiella pneumoniae

Like *E. coli*, bacteria of the species *K. pneumoniae* are frequent colonizers of the gut in humans, particularly those with a history of hospitalization, and other vertebrates. Infections with *K. pneumoniae*:

- are particularly common in hospitals among vulnerable individuals such as preterm infants and patients with impaired immune systems, diabetes or alcohol-use disorders and those receiving advanced health care;

- are commonly urinary and respiratory tract infections and, among neonates, bloodstream infections;

- are a common cause of gram-negative bloodstream infections; and

- can spread readily between patients, leading to nosocomial outbreaks, which frequently occurs in intensive care units and neonatal care facilities.

The mortality rates for hospital-acquired *K. pneumoniae* infections depend on the severity of the underlying condition, even when people are treated with appropriate antibacterial drugs.

Salmonella spp.

Salmonella spp.:

- is a major cause of foodborne illness throughout the world;

- is a zoonotic pathogen and can thus be found in the intestines of many food-producing animals such as poultry and pigs; infection is usually acquired by consumption of contaminated water or food of animal origin, such as mainly undercooked meat, poultry, eggs and milk;

- can also contaminate the surface of fruits and vegetables through contact with human or animal faeces, which can lead to foodborne outbreaks; and

- mostly causes gastroenteritis, while some strains, particularly *Salmonella enterica* serotypes Typhi and Paratyphi, are more invasive and typically cause enteric fever – a more serious infection that poses problems for treatment due to antibiotic-resistant strains in many parts of the world.

CAESAR focuses on nontyphoidal *Salmonella*, because these are main diarrhoeal pathogens transmitted via the food chain. In many countries, the incidence of nontyphoidal *Salmonella* infections has increased markedly in recent years, for reasons that are unclear. One estimate suggests that there are around 94 million cases, resulting in 155 000 deaths, of nontyphoidal *Salmonella* gastroenteritis each year. The majority of the disease burden, according to this study, is in the WHO South-East Asian Region and the WHO Western Pacific Region *(3)*.

Pseudomonas aeruginosa

P. aeruginosa:

- is a non-fermentative gram-negative bacterium that is ubiquitous in aquatic environments in nature;

- is an opportunistic pathogen for plants, animals and humans and is a major and dreaded cause of infection among hospitalized patients with localized or systemic impairment of immune defences;

- commonly causes hospital-acquired pneumonia (including ventilator-associated pneumonia) and bloodstream and urinary tract infections;

- is difficult to control in hospitals and institutional environments, because of its ubiquity, enormous versatility and intrinsic tolerance to many detergents, disinfectants and antimicrobial compounds;

- causes bacterial complications among people with cystic fibrosis, leading to chronic colonization and intermittent exacerbation of the condition with, for example, bronchiolitis and acute respiratory distress syndrome; and

- is common in burn units, where eradicating colonizing strains with classic infection control procedures is almost impossible.

Acinetobacter spp.

The *Acinetobacter* genus comprises many species that can be roughly divided between the *Acinetobacter baumannii* group (consisting of the species *A. baumannii, A. pittii* and *A nosocomialis*) and the *Acinetobacter*

non-*baumannii* group (consisting of many environmental species with low pathogenicity). Species belonging to the *A. baumannii* group:

- have been identified as pathogens in nosocomial pneumonia (particularly ventilator-associated pneumonia), central line-associated bloodstream infections, urinary tract infections, surgical site infections and other types of wound infection;

- are not considered ubiquitous in nature, in contrast to many species of the *Acinetobacter* genus; and

- have low carrying rates on the skin and in the faeces.

Risk factors for infection with the *A. baumannii* group include advanced age, the presence of serious underlying diseases, immune suppression, major trauma or burn injuries, invasive procedures, presence of indwelling catheters, mechanical ventilation, extended hospital stay and previous administration of antimicrobial agents. The risks for acquiring a multidrug-resistant strain of the *A. baumannii* group are similar and also include prolonged mechanical ventilation, prolonged intensive care unit or hospital stay, exposure to infected or colonized patients, increased frequency of interventions, increased disease severity and receipt of broad-spectrum antimicrobial agents, especially third-generation cephalosporins, fluoroquinolones and carbapenems.

Staphylococcus aureus

S. aureus:

- is a gram-positive bacterium that can be part of the normal microbiota on the skin and in the nose but is another of the most important human pathogens;

- can cause a variety of infections – most notably skin, soft tissue, bone and bloodstream infections – and is also the most common cause of postoperative wound infections; and

- produces toxic factors (some strains) that can cause a variety of specific symptoms, including toxic shock syndrome and food poisoning.

Several successful *S. aureus* clones are responsible for most of the international spread and outbreaks in health care and community settings. A recent structured survey showed that the most prevalent clones among methicillin-resistant *S. aureus* (MRSA) in European Union countries are ST22 (EMRSA15), ST225 (New York/Japan), ST8 (US300), ST5 (New York/Japan) and ST8 (South German) *(4)*. Among methicillin-susceptible *S. aureus,* the most prevalent clones are ST7, ST15, ST5, ST45 and ST8. The clonal structure of MRSA and methicillin-susceptible *S. aureus* in the countries participating in CAESAR remains to be determined.

Streptococcus pneumoniae

S. pneumoniae:

- is the leading cause of community-acquired pneumonia worldwide, which is among the leading causes of death of children under 5 years of age;

- causes other common, mild, self-limiting infections such as acute otitis media but also extend to cases of invasive disease with high mortality such as meningitis;

- is associated with the highest case-fatality rate among the bacterial causes of meningitis, and is the most likely to leave survivors with permanent residual symptoms;

- caused about 826 000 deaths (582 000–926 000) among children aged 1–59 months; the clinical burden of pneumococcal infection is concentrated among the oldest and youngest sections of the population; for HIV-negative children, pneumococcal infection corresponds to 11% of all deaths in this age group *(5)*; and

- is commonly found as asymptomatic nasopharyngeal carriage, where the prevalence varies by age and region; the asymptomatic carriage state is responsible for much of the transmission within populations, such as in childcare centres.

Enterococcus faecium and *Enterococcus faecalis*

Enterococcus spp.:

- belong to the normal bacterial microbiota of the gastrointestinal tract of both humans and other animals and are usually low-pathogenic but can cause invasive disease under certain circumstances;

- can act as true pathogens and not only as opportunistic commensals, as recently high-risk clones were recognized;

- can cause a variety of infections, including endocarditis, bloodstream and urinary tract infections, and are associated with peritonitis and intra-abdominal abscesses;

- cause 3–4 nosocomial bloodstream infections per 10 000 hospital discharges in the United States of America and contribute to increasing mortality as well as additional hospital stay;

- emerge as important nosocomial pathogens, as documented in epidemiological data collected over the last two decades, and exemplified by the expansion of a major hospital-adapted polyclonal subcluster clonal complex 17 (CC17) in *E. faecium* and by CC2 and CC9 in *E. faecalis*, with the latter clones isolated from farm animals; and

- are highly tenacious and thus easily disseminate in the hospital setting and infections caused by resistant strains are difficult to treat.

E. faecalis and *E. faecium* cause the vast majority of clinical enterococci infections in humans. The emergence of particular clones and clonal complexes of *E. faecalis* and *E. faecium* was paralleled by increases in resistance to glycopeptides and high-level resistance to aminoglycosides. These two antimicrobial classes represent the few remaining therapeutic options for treating human infections caused by penicillin-resistant *E. faecium*.

References

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ANNEX F