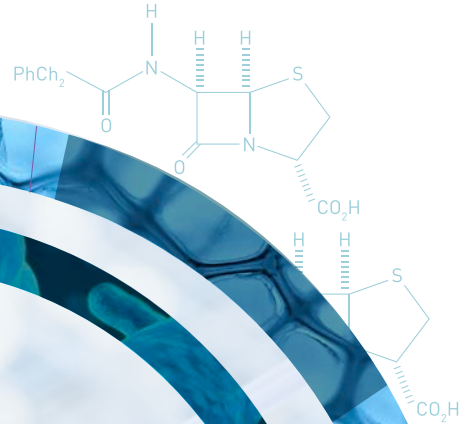




World Health
Organization

REGIONAL OFFICE FOR Europe



Central Asian and
Eastern European
Surveillance of
**Antimicrobial
Resistance**

*Annual report
2014*

Central Asian and
Eastern European
Surveillance of
**Antimicrobial
Resistance**

*Annual report
2014*

Abstract

This report describes the resistance data from five countries in the WHO European Region gathered through the Central Asian and Eastern European Surveillance of Antimicrobial Resistance network. Guidance is provided to the reader on how to interpret the surveillance data with caution, taking conditions outside the direct control of the national antimicrobial resistance surveillance system into account, which may reduce the reliability and representativeness of the data. The aim of this report is to provide guidance and inspiration to countries that are building or strengthening their national antimicrobial resistance surveillance and to stimulate the sharing of data internationally. WHO and its partners remain committed to support countries in these endeavours through the activities of the network.

Keywords

ANTIMICROBIAL RESISTANCE
NATIONAL SURVEILLANCE NETWORKS
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EXTERNAL QUALITY ASSESSMENT

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Foreword

In September 2011, all 53 countries of the WHO European Region adopted the European strategic action plan on antibiotic resistance. The action plan was developed recognizing that in many countries in the Region, antibiotic resistance had been neglected, no systematic surveillance of antibiotic use and resistance was in place, control efforts needed to be coordinated between the health and other relevant sectors, antibiotic resistance can spread internationally through travel and trade, and international standards and mechanisms for sharing data and information were needed.

The action plan contains seven strategic objectives, intended to comprehensively cover the complex factors related to bacterial resistance. Surveillance of antibiotic use and resistance are considered the backbone of the action plan, as they are necessary in order to document the extent of the problem, follow the emergence of and trends in specific pathogen–agent combinations and evaluate the effectiveness of targeted interventions.

Within the Region, large differences can be found in terms of infrastructure, awareness and actions taken against antimicrobial resistance (AMR). For example, surveillance of antibiotic resistance is undertaken by all countries of the European Union, as well as Iceland, Liechtenstein and Norway, via the European Antimicrobial Resistance Surveillance Network of the European Commission coordinated by the European Centre for Disease Prevention and Control (ECDC), whereas surveillance of and information about antibiotic resistance has been scattered and incomplete in the non-European Union Member States of the WHO European Region.

To address this discrepancy, the WHO Regional Office for Europe, together with the European Society of Clinical Microbiology and Infectious Diseases and the Netherlands National Institute for Public Health and the Environment, established the Central Asian and Eastern European Surveillance of Antimicrobial Resistance (CAESAR) network in 2012 to assist countries in setting up or strengthening national AMR surveillance and to contribute to region-wide AMR surveillance. These efforts are closely coordinated with ECDC to ensure that data are comparable and compatible, which will provide a pan-European overview of trends and sources of AMR, guide national and international targeted actions and measure their effectiveness. Compatibility between the two surveillance networks also ensures a smooth transition for European Union candidate countries from the WHO system to ECDC upon accession.

This report describes the first resistance data from five countries in the WHO European Region gathered through the CAESAR network. Guidance is provided to the reader on how to interpret the surveillance data with caution, taking conditions outside the direct control of the national AMR surveillance system into account, which may reduce the reliability and representativeness of the data.

The aim of this report is to provide guidance and inspiration to countries that are building or strengthening their national AMR surveillance and to stimulate the sharing of data internationally. WHO and its partners remain committed to support countries in these endeavours through the activities of the CAESAR network.

Guenael R. Rodier, MD

Director, Division of Communicable Diseases, Health Security and Environment
WHO Regional Office for Europe



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Belarus

Professor Leonid Titov, National AMR Focal Point; Head, Laboratory for Clinical and Experimental Microbiology, Republican Research and Practical Center for Epidemiology and Microbiology, Minsk

Dr Alexander Davydov, Republican Research and Practical Center for Epidemiology and Microbiology, Minsk

Serbia

Professor Zora Jelesic, National AMR Focal Point; Head, Center for Microbiology, Institute for Public Health of Vojvodina, Novi Sad

Switzerland

Dr Andreas Kronenberg, National AMR Focal Point, Institute for Infectious Diseases, University of Bern, Bern

The former Yugoslav Republic of Macedonia

Dr Golubinka Bosevska, National AMR Focal Point; Head, Laboratory for Virology and Molecular Diagnostics, Institute of Public Health, Skopje

Dr Biljana Kakaraskoska Boceska, Institute of Public Health, Skopje

Professor Zaklina Cekovska, Medical faculty, Skopje

Associate Professor Ana Kaftandzieva, Medical faculty, Skopje

Professor Nikola Panovski, Medical faculty, Skopje

Turkey

Dr Husniye Simsek, National AMR Focal Point, Microbiology Reference Laboratories Department, Public Health Institution of Turkey, Ankara

WHO would like to thank all national AMR focal points for their active participation in CAESAR and looks forward to including their data in future editions of this report.

WHO would also like to thank the pool of experts and WHO Country offices for their support in setting up and strengthening national AMR surveillance in the countries, and looks forward to the continued collaboration.

WHO would like to thank United Kingdom National External Quality Assessment Service for Microbiology of Public Health England for organizing the CAESAR External Quality Assessment.

About the authors



National Institute for Public Health
and the Environment
Ministry of Health, Welfare and Sport

WHO Collaborating Centre for AMR Epidemiology and Surveillance at the National Institute for Public Health and the Environment, the Netherlands

Dr Tjalling Leenstra, Head, WHO Collaborating Centre of AMR Epidemiology and Surveillance; CAESAR Contact Point

Mr Jos Monen, Data Manager; CAESAR International Data Manager

Ms Sjoukje Woudt, Epidemiologist

Dr Wieke Altorf-van der Kuil, Epidemiologist



WHO Regional Office for Europe

Dr Nienke van de Sande-Bruinsma, Technical Officer, AMR; CAESAR Coordinator

Dr Danilo Lo Fo Wong, Programme Manager, AMR

Dr Saskia Nahrgang, Technical Officer, AMR



European Society of Clinical Microbiology and Infectious Diseases

Dr Christian G. Giske, Senior Lecturer and Senior Consultant Physician, Department of Clinical Microbiology, Karolinska University Hospital, Stockholm, Sweden

Dr Robert Skov, Head of Bacteriologic Surveillance and Infection Control, Statens Serum Institut, Copenhagen, Denmark

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Abbreviations

AMR	antimicrobial resistance
AST	antibiotic susceptibility testing
BSAC	British Society for Antimicrobial Chemotherapy
CAESAR	Central Asian and Eastern European Surveillance of Antimicrobial Resistance
CC	clonal complex
CLSI	Clinical and Laboratory Standards Institute
CSF	cerebrospinal fluid
EARS-Net	European Antimicrobial Resistance Surveillance Network
ECDC	European Centre for Disease Prevention and Control
EQA	external quality assessment
ESBL	extended-spectrum beta-lactamase
ESCMID	European Society of Clinical Microbiology and Infectious Diseases
ESGARS	ESCMID Study Group for Antimicrobial Resistance Surveillance
EU	European Union
EUCAST	European Committee on Antimicrobial Susceptibility Testing
IBVPD	WHO Global Invasive Bacterial Vaccine Preventable Diseases Laboratory Network
ICU	intensive care unit
I	intermediate
I+R	intermediate or resistant
R	resistant
S	susceptible
ISO	International Organization for Standardization
MIC	minimum inhibitory concentration
MRSA	methicillin-resistant <i>Staphylococcus aureus</i>
MSSA	methicillin-sensitive <i>Staphylococcus aureus</i>
PBP	penicillin-binding protein
RIVM	National Institute for Public Health and the Environment, the Netherlands
UK NEQAS	United Kingdom National External Quality Assessment Service for Microbiology
VEB	Vietnamese extended-spectrum beta-lactamase
WHONET	WHO microbiology laboratory database software

Summary

The CAESAR network is a joint initiative of the WHO Regional Office for Europe, the European Society of Clinical Microbiology and Infectious Diseases and the Dutch National Institute for Public Health and the Environment. CAESAR aims to support all countries of the WHO European Region that are not part of the European AMR Surveillance Network coordinated by ECDC in the European Union.

Currently, Albania, Armenia, Azerbaijan, Belarus, Bosnia and Herzegovina, Georgia, Kyrgyzstan, Montenegro, the Republic of Moldova, the Russian Federation, Serbia, Switzerland, Tajikistan, the former Yugoslav Republic of Macedonia, Turkey, Turkmenistan and Uzbekistan, as well as Kosovo (in accordance with United Nations Security Council resolution 1244 (1999)), are engaged at various stages of development and participation in CAESAR. To date, five countries (Belarus, Serbia, Switzerland, the former Yugoslav Republic of Macedonia and Turkey) have submitted data to the CAESAR database.

This is the first annual CAESAR report describing the data of the initial five Member States that reported antimicrobial susceptibility testing results of invasive isolates, and background information about patients from their respective national AMR surveillance networks.

CAESAR collects AST data from blood and cerebrospinal fluid for eight bacterial species of public health and clinical importance: *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Acinetobacter species*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Enterococcus faecalis* and *Enterococcus faecium*. The trends of resistance observed among these pathogens reported by the first five countries are presented in Chapter 6. In some countries, limiting conditions outside the direct control of the national AMR surveillance system may reduce the reliability and representativeness of the data because they influence the selection of patients eligible for blood culturing or the quality of antimicrobial susceptibility testing performed. Therefore this report includes a reader's guide that describes several sources of error and bias in AMR surveillance data (Chapter 5, Annex 1). To further guide the interpretation of the data presented in this report, the authors and the national AMR focal points of the five countries have judged the level of evidence for each country-specific data chapter.

Due to differences in the level of evidence reported by the countries, this first CAESAR annual report does not make comparisons between the resistance levels observed in the countries. In Chapter 6, the results of each country are displayed and discussed separately.

In Chapter 7, the results of the first CAESAR external quality assessment exercise are displayed. Eight countries or areas from the CAESAR network participated. All participating laboratories (n=120) followed international guidelines, namely from the Clinical and Laboratory Standards Institute (88%) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) or EUCAST-related (14%). Overall, performance was generally very good and consistent with that seen in previous external quality assessment surveys among participants in European Union countries. External quality assessment is a valuable tool in the quality assurance of antimicrobial susceptibility testing and gives an indication of the validity of comparing collated data between laboratories in resistance surveillance studies.

In conclusion, this report is meant to provide guidance to countries that are building or strengthening their national AMR surveillance. Even though, for some of the countries, the data displayed in this report should be interpreted with caution, the high resistance levels displayed confirm the need for action, and emphasize the importance of good clinical practice in diminishing the further development of AMR. Using surveillance data to provide treatment guidance to physicians, as well as to increase awareness among policy-makers and the public, is essential in fighting AMR.

CHAPTER
1

Introduction

1.1 AMR threat

The discovery of antibiotics and other antimicrobials has dramatically changed human and veterinary medicine, preventing and curing infections, and saving millions of lives. Through the natural process of adaptation, bacteria and other microorganisms eventually become resistant to antimicrobial treatment. However, the rate at which resistance emerges is greatly accelerated by the overuse and misuse of antimicrobials. As currently available antimicrobials lose their effectiveness, and only very few new drugs are being developed, many types of infection are becoming life threatening again and surgical procedures hazardous (1).

1.2 European strategic action plan on antibiotic resistance

The WHO European Region covers 53 countries, including those of the European Union (EU), the Balkans, the Caucasus, central Asia and the Russian Federation. Building on the momentum created by World Health Day 2011, all 53 countries adopted a new European strategic action plan on antibiotic resistance in September 2011, in Baku, Azerbaijan, at the 61st session of the WHO Regional Committee for Europe, which aims to preserve the ability of modern medicine to prevent and treat infections for this and future generations (2).

Following extensive consultation with experts and policy-makers, the action plan calls upon strong national coordination between relevant sectors and contains seven strategic objectives. The plan provides guidance to national governments to address the complex factors that relate to bacterial resistance and its driver, antibiotic usage (especially overuse and misuse). It identifies key areas where action must be taken to ensure that Europeans are safe, including surveillance of AMR and consumption, infection prevention and control, innovation and research, prevention of AMR in the veterinary and agricultural sectors, and awareness raising. The resolution accompanying the action plan urges Member States to ensure political commitment and resources for its implementation. The WHO Regional Office for Europe and its partners are working together with Member State governments to implement this comprehensive strategic action plan.

1.3 Strengthen surveillance of AMR

AMR is a global problem and no single country can control it alone. The interconnectivity of countries and the globalization of travel and trade increase the risk of spreading bacteria or genes that jeopardize effective treatment or the prevention of bacterial infections in every corner of the world. Still, the problem of AMR has been and remains neglected in many countries, in part because it is not properly documented through systematic monitoring or surveillance systems. To combat AMR effectively, information is needed on antimicrobial use, as well as the origin and spread of resistant pathogens and their impact on society.

The situation in the Region is quite unique as approximately half of the Region has well established national and international surveillance systems (e.g. EU) whereas the other half does not. As resistant bacteria do not respect geographical (or biological) borders, the lack of surveillance in Eastern Europe and Central Asia are of particular concern to WHO. Harmonized and coordinated surveillance networks in all countries in the Region are key to protect health from a cross-border threat.



CHAPTER
2

CAESAR

2.1 Objectives

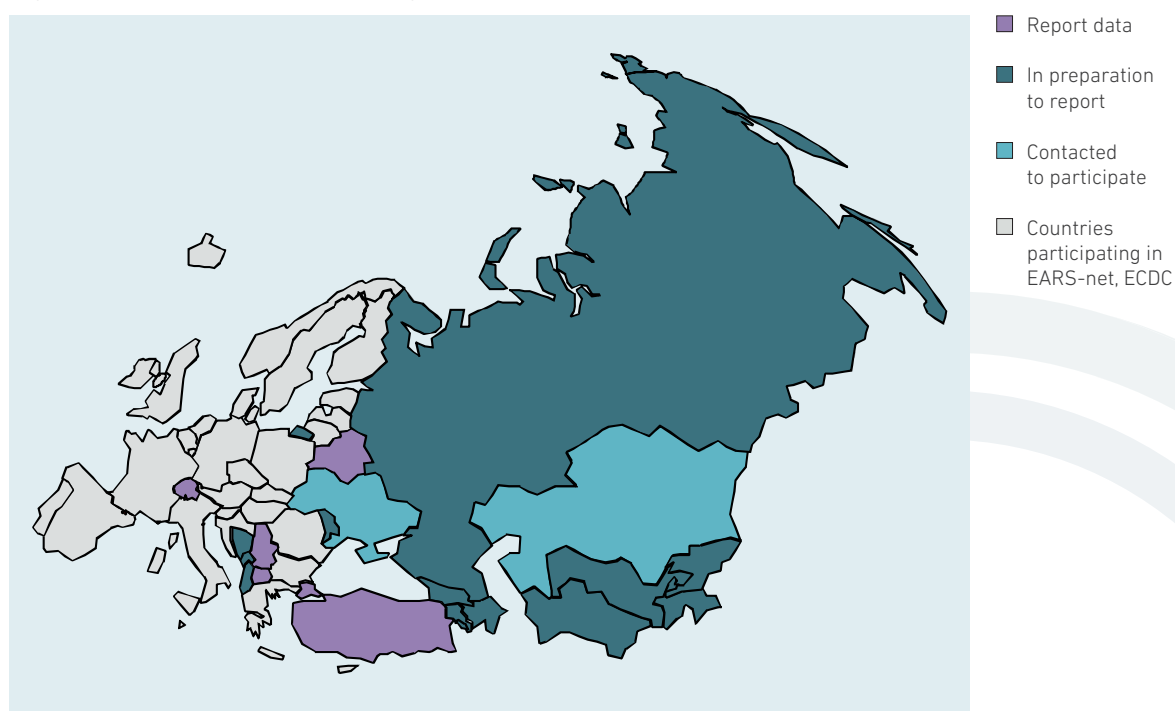
The CAESAR network is a joint initiative of the Regional Office, the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) and the National Institute for Public Health and the Environment, the Netherlands (RIVM). CAESAR aims to support all countries of the Region that are not part of the European AMR Surveillance Network (EARS-Net) coordinated by ECDC in the EU.

The aim of CAESAR is to gradually set up a network of national surveillance systems for AMR, and to enable countries to strengthen their AMR surveillance, as well as laboratory capacity and quality. In order to complement the data obtained for the EU through EARS-Net and enable comparison of data throughout the whole Region, the methodology used in CAESAR (WHO Regional Office for Europe; unpublished observations, 11 May 2015) is closely coordinated with ECDC (3). These efforts will provide a pan-European overview of trends and sources of AMR, guide national and international targeted actions and measure their effectiveness.

2.2 Participating countries

At present, Albania, Armenia, Azerbaijan, Belarus, Bosnia and Herzegovina, Georgia, Kyrgyzstan, Montenegro, the Republic of Moldova, the Russian Federation, Serbia, Switzerland, Tajikistan, the former Yugoslav Republic of Macedonia, Turkey, Turkmenistan, Ukraine and Uzbekistan, as well as Kosovo (in accordance with United Nations Security Council resolution 1244 (1999)), are engaged in CAESAR at various stages of development and participation. Currently, five countries (Belarus, Serbia, Switzerland, the former Yugoslav Republic of Macedonia and Turkey) have submitted data to the CAESAR database (Fig. 1), which are presented in this report.

Fig. 1. Status of countries reporting AMR data to the CAESAR database



2.3 Challenges

As an integral part of the implementation of the European strategic action plan on antibiotic resistance, the Regional Office and experts performed country situation analyses in a number of countries. The main challenges and needs for AMR surveillance observed during country visits are:

- limited human and financial resources to address the need for laboratory capacity building;
- continuing need to educate laboratory personnel;
- the need for implementation of updated guidelines on the standardization of antibiotic susceptibility testing (AST) (from the Clinical and Laboratory Standards Institute (CLSI) and EUCAST), laboratory methods for species identification and blood culturing;
- the need for standard operating procedures and quality control in laboratory practice;
- the need to improve sampling habits and utilization of medical microbiologic diagnostics in hospitals; and
- the need to improve laboratory information management and setting up an infrastructure for central data collection at a national reference laboratory.

The Regional Office and its partners organize technical workshops to support countries in meeting these challenges. So far, two rounds of the annual CAESAR external quality assessment (EQA) of AST have been performed by laboratories in countries engaged in CAESAR. These were conducted by the United Kingdom National EQA Service for Microbiology (UK NEQAS). EQA of AST in diagnostic laboratories is a valuable tool for validity, enabling comparison of data between laboratories. In addition, WHO is raising funds and seeking more additional technical support from partners to provide more in-depth capacity building and to set up twinning activities in the near future. The main objective of the twinning activities is to establish sustainable links between experienced and less-experienced AMR reference laboratories to exchange knowledge, skills and experience.

2.4 Steps towards participation

The first step towards participation in CAESAR and the implementation of the European strategic action plan on antibiotic resistance in general, is the appointment of an AMR focal point in each country, territory or area outside the EU, who should play a leading role in the formation of a coordinating committee for the containment of AMR (Table 1). The committee should identify key areas where action must be taken, and develop or update the strategic action plan on AMR. One of these key areas is AMR surveillance.

The Regional Office offers a country situation analysis, in collaboration with ESCMID and RIVM, to determine the country status regarding prevention and control of AMR through surveillance, prudent use of antimicrobials and infection control, specifically focusing on promoting national coordination and strengthening surveillance of antimicrobial consumption and resistance. An assessment report is provided to the WHO country office and the health ministry containing observations and recommendations for further action, which, for example, includes country workshops to support setting up or strengthening a national AMR surveillance system. Workshop topics, depending on the needs of the country, include:

- CAESAR methodology, data collection (among others, WHONET)¹ and data analysis;
- an introduction to EUCAST guidelines and interpretation of AST data; and

¹ WHO microbiology laboratory database software.

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

A pool of experts supports WHO in providing technical support to Member States for the implementation of the European strategic action plan (Table 2). One part of this is to provide technical support to set up and strengthen national AMR surveillance systems via multicountry and national AMR workshops and consultancies.

Table 1. AMR focal points in countries, territories and areas of the CAESAR network

Country, territory or area	AMR focal point
Albania	Dr Lindita Molla (Food Safety and Nutrition, Department of Environment and Public Health, Institute of Public Health)
Armenia	Dr Kristina Gyurjyan (Head, Public Health Department, Ministry of Health)
Azerbaijan	Dr Nazifa Mursalova (Sector of Sanitary Epidemiological Surveillance, Ministry of Health)
Belarus	Dr Vladimir Gorbunov (Director, Republican Research and Practical Center for Epidemiology and Microbiology) Professor Leonid Titov (Head, Laboratory for Clinical and Experimental Microbiology, Republican Research and Practical Center for Epidemiology and Microbiology)
Bosnia and Herzegovina	Professor Mirsada Hukic (Clinical Microbiology Department, Clinical Center University of Sarajevo) Dr Amela Dedeic-Ljubovic (Head, Clinical Microbiology Department, Clinical Center University of Sarajevo) Dr Pava Dimitrijevic (Head, Department of Microbiology, Public Health Institute of the Republic of Srpska)
Georgia	Dr Paata Imnadze (Deputy Director, National Center for Disease Control and Public Health)
Kazakhstan	National AMR focal point pending nomination
Kyrgyzstan	Dr Baktygul Ismailova (Chief Specialist, Public Health Department, Ministry of Health)
Montenegro	Professor Gordana Mijovic (Centre for Medical Microbiology, Institute of Public Health)
Republic of Moldova	Dr Radu Cojocar (Director, Laboratory, Division for Surveillance of Highly Dangerous Pathogens and Bio-security, National Centre for Public Health, Ministry of Health)
Russian Federation	Professor Roman S. Kozlov (Director, Institute of Antimicrobial Chemotherapy, Smolensk State Medical Academy), Chief Specialist of MoH of Russian Federation on Clinical Microbiology and Antimicrobial Resistance
Serbia	Professor Zora Jelesic (Head, Center for Microbiology, Institute for Public Health of Vojvodina)
Switzerland	Dr Andreas Kronenberg (Swiss Centre for Antibiotic Resistance, Institute for Infectious Diseases, University of Bern)
Tajikistan	Dr Azamjon Safolovich Mirzoev (Deputy Head, State Sanitary Epidemiology Surveillance Service, Ministry of Health and Social Protection of the Population)
The former Yugoslav Republic of Macedonia	Dr Golubinka Bosevska (Head, Laboratory for Virology and Molecular Diagnostics, Institute of Public Health)
Turkey	Dr Husniye Simsek (Microbiology Reference Laboratories Department, Public Health Institution of Turkey)
Turkmenistan	Dr Gurbangul Ovylyakulova (Head, Acute Dangerous Infections Control, State Sanitary Epidemiology Service, Ministry of Health and Medical Industry)
Ukraine	Professor Aidyn Salmanov Hurban Ogly (Head, Department of Microbiology and Epidemiology, Shupyk National Medical Academy of Postgraduate Education)
Uzbekistan	Dr Gulnora Abdukhalilova (Head, Laboratory, Research Institute of Epidemiology, Microbiology and Infectious Diseases)
Kosovo ^a	Dr Lul Raka (Medical Microbiologist, Kosovo Institute of Public Health)

^a In accordance with the United Nations Security Council resolution 1244 (1999).

Table 2. Pool of international experts providing CAESAR support to Member States, 2012–2014

Country/Project group	Expert	Institute
CAESAR project group	Dr Christian G. Giske Dr Tjalling Leenstra Dr Danilo Lo Fo Wong Mr Jos Monen Dr Saskia Nahrgang Dr Robert Skov Dr Nienke van de Sande-Bruinsma	ESCMID Study Group for AMR Surveillance (ESGARS) RIVM, WHO Collaborating Centre for AMR Epidemiology and Surveillance WHO Regional Office for Europe RIVM, WHO Collaborating Centre for AMR Epidemiology and Surveillance WHO Regional Office for Europe ESGARS WHO Regional Office for Europe
Croatia	Dr Iva Butic Professor Arjana Tambic	University Hospital for Infectious Diseases, Zagreb
Hungary	Dr Bela Kocsis	Semmelweis University, Budapest
Netherlands	Mr Rob Riesmeijer Dr Mariken van der Lubben	RIVM, WHO Collaborating Centre for AMR Epidemiology and Surveillance
Poland	Professor Waleria Hryniewicz	National Medicines Institute, Warsaw
Slovenia	Professor Katja Seme	University of Ljubljana
Sweden	Dr Oskar Ekelund	EUCAST Development Laboratories
Turkey	Dr Osman Cirit Dr Onur Karatuna	Gaziantep Dr Ersin Arslan State Hospital Acibadem University School of Medicine, Istanbul
United Kingdom	Dr Christine Walton Professor Neil Woodford	Public Health England, WHO Collaborating Centre for Reference & Research on AMR and Healthcare Associated Infections

2.5 Progress 2012–2014

Table 3 provides an overview of the current implementation status of CAESAR in countries.

Table 3. Overview of progress on CAESAR related activities

Country or area	National AMR focal point appointed	Intersectoral coordinating mechanism to contain AMR set up	National AMR action plan developed	National AMR reference laboratory in place	National AMR surveillance in place	AMR data reported to CAESAR	Subset of laboratories participate in CAESAR EQA	National AMR workshop held
Albania	✓	✓	✗	✗	✗	⚙️	✓	✗
Armenia	✓	✓	✓	⚙️	✗	⚙️	✓	✗
Azerbaijan	✓	✗	✗	✗	✗	✗	✓	✗
Belarus	✓	✗	⚙️	✓	✓	✓	✓	✓
Bosnia and Herzegovina	✓	✓	✗	✗	✗	⚙️	✓	⚙️

Country or area	National AMR focal point appointed	Intersectoral coordinating mechanism to contain AMR set up	National AMR action plan developed	National AMR reference laboratory in place	National AMR surveillance in place	AMR data reported to CAESAR	Subset of laboratories participate in CAESAR EQA	National AMR workshop held
Georgia	✓	✓	✓	✓	✗	⚙️	✓	✓
Kazakhstan	✗	✗	✗	✗	✗	✗	✗	✗
Kyrgyzstan	✓	✗	✗	✗	✗	✗	✓	✗
Montenegro	✓	✓	✓	⚙️	✗	⚙️	✓	✗
Republic of Moldova	✓	✓	✗	✓	✓	⚙️	✓	✓
Russian Federation	✓	✓	✗	✓	✓	⚙️	✓	✗
Serbia	✓	⚙️	✗	✓	✓	✓	✓	✓
Switzerland	✓	✓	✗	✓	✓	✓	✗	✗
Tajikistan	✓	✗	✗	✗	✗	✗	✗	✗
The former Yugoslav Republic of Macedonia	✓	✓	✓	✓	✓	✓	✓	✓
Turkey	✓	✓	✓	✓	✓	✓	✓	✓
Turkmenistan	✓	✗	✗	✗	✗	⚙️	✗	✗
Ukraine	✓	✗	✗	✓	✗	✗	✗	✗
Uzbekistan	✓	✗	⚙️	⚙️	✗	⚙️	✗	✓
Kosovo ^a	✓	✗	✗	✗	✓	⚙️	✓	✗

^a In accordance with the United Nations Security Council resolution 1244 (1999).

- ✓ Yes
- ✗ No
- ⚙️ In progress



CHAPTER

3

Data collection and analysis

3.1 Data collection procedures

CAESAR collects susceptibility test results of invasive isolates and background information about patients from national AMR surveillance networks following a data request to the national AMR focal point. The data is prepared by the national data manager and transferred electronically to the CAESAR international data manager at RIVM. The national AMR focal point and national data manager are responsible for collecting data from the laboratories in the national surveillance network. Network laboratories are asked to report antimicrobial susceptibility results for the first isolate from blood or cerebrospinal fluid (CSF) per patient per year, including additional isolate and patient information for a pre-specified number of bacterial species and antimicrobial agents. Data are collected and compiled according to the specifications of the CAESAR exchange format (WHO Regional Office for Europe, unpublished observations, 11 May 2015), which is compatible with the EARS-Net format (3).

CAESAR collects AST data for eight bacterial species of public health and clinical importance:

- *Escherichia coli*
- *Klebsiella pneumoniae*
- *Pseudomonas aeruginosa*
- *Acinetobacter species*
- *Staphylococcus aureus*
- *Streptococcus pneumoniae*
- *Enterococcus faecalis*
- *Enterococcus faecium*.

The CAESAR manual contains a panel of antimicrobials (WHO Regional Office for Europe, unpublished report, 11 May 2015), recommended by EUCAST and ESGARS to detect resistance mechanisms. Other antimicrobials are collected as well but not analysed.

Once data are submitted to CAESAR, data are analysed, and results are reported back to the AMR focal point by a standardized feedback report. This feedback report gives the proportion of resistance for the important antimicrobial groups, as well as information on pathogens with important or unusual resistance patterns, and information on the validity and completeness of the data. Subsequently, the AMR focal point is asked to verify the results and, if needed, update the data. After approval, the data are added to the CAESAR database.

In addition to the bacterial species listed in the CAESAR manual, countries are encouraged to include pathogen-antibiotic combinations in their surveillance system that are of national concern or relevance.

3.2 Analysis

AST results are presented as the proportion of isolates of a particular microorganism that is resistant (R) or non-susceptible intermediate and resistant (I+R) to a specific antimicrobial agent; for example, the number of *E. coli* resistant to ciprofloxacin divided by the total number of *E. coli* in which susceptibility to ciprofloxacin was tested.

The R and I+R interpretations are based on clinical breakpoint criteria used by local laboratories. CAESAR encourages countries to adopt national standards for AST and promotes the use of internationally accepted guidelines like EUCAST and CLSI. If fewer than 30 AST results for a specific microorganism-antimicrobial combination were submitted, the results are marked, indicating that they should be interpreted with caution.



CHAPTER

4

Pathogens under CAESAR surveillance

The following text on pathogens under CAESAR surveillance was adopted from the *AMR: global report on surveillance 2014* published by WHO (4) and the EARS-Net annual report published by ECDC in 2014 (5).

4.1 *E. 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- and hospital-acquired urinary tract infections (including pyelonephritis);
- the most frequent cause of bloodstream infection in people of all ages;
- associated with intra-abdominal infections such as peritonitis;
- a cause of meningitis in neonates; and
- one of the leading causative agents of foodborne infections worldwide.

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

4.1.1 Evolution of AMR in *E. coli*

Resistance in *E. coli* readily develops either through mutation, which is often the case for fluoroquinolone resistance, or by acquisition of mobile genetic elements, which has been the case for broad-spectrum penicillins (e.g. ampicillin or amoxicillin) and resistance to third-generation cephalosporins and carbapenems. Resistance to third-generation cephalosporins is mainly conferred by enzymes known as extended-spectrum beta-lactamases (ESBLs); these enzymes degrade many beta-lactam drugs. ESBLs are transmissible between bacteria and even between bacterial species. Because *E. coli* strains that have ESBL are generally also resistant to several other antibacterial drugs, carbapenems and piperacillin-tazobactam remain the only available treatment option for severe infections. A recently emerging threat is carbapenem resistance in *E. coli* mediated by a range of carbapenemases, which confer resistance to virtually all available beta-lactam antibacterial drugs.

4.2 *K. 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 medical care. Most common are urinary and respiratory tract infections and, in neonates, bloodstream infections. *K. pneumoniae* is a common cause of Gram-negative bloodstream infections. Like other bacteria in health care settings, *K. pneumoniae* can spread readily between patients, leading to nosocomial outbreaks.

This frequently occurs in intensive care units (ICUs) and neonatal care facilities. The mortality rates for *K. pneumoniae* hospital-acquired infections depend on the severity of the underlying condition, even when treated with appropriate antibacterial drugs.

4.2.1 Evolution of AMR in *K. pneumoniae*

Similar to *E. coli*, *K. pneumoniae* acquires resistance to multiple antibacterial drugs mainly through horizontal transfer of mobile genetic elements such as transposons or plasmids. In contrast to *E. coli*, *K. pneumoniae* carries a resistance gene (chromosomally located beta-lactamase) that naturally renders ineffective penicillins with an extended spectrum, such as ampicillin and amoxicillin. Resistance to other widely used and available oral antibacterial drugs such as cotrimoxazole and fluoroquinolones (e.g. ciprofloxacin) has emerged and spread globally. Thus, few options remain for oral treatment of *Klebsiella* infections in many parts of the world. ESBLs and carbapenemases are found to a higher extent in *K. pneumoniae* than in *E. coli*.

4.3 *P. aeruginosa*

P. aeruginosa is a non-fermentative Gram-negative bacterium that is ubiquitous in aquatic environments in nature. It is an opportunistic pathogen for plants, animals and humans, and is a major and dreaded cause of infection in hospitalized patients with localized or systemic impairment of immune defences. It commonly causes hospital-acquired pneumonia (including ventilator-associated pneumonia), bloodstream and urinary tract infections. Because of its ubiquity, enormous versatility and intrinsic tolerance to many detergents, disinfectants and antimicrobial compounds, it is difficult to control *P. aeruginosa* in hospitals and institutional environments. In patients with cystic fibrosis, *P. aeruginosa* causes severe bacterial complication leading to chronic colonization and intermittent exacerbation of the condition with, for example, bronchiolitis and acute respiratory distress syndrome. Finally, *P. aeruginosa* is commonly found in burn units, where it is almost impossible to eradicate colonizing strains with classic infection control procedures.

4.3.1 Evolution of AMR in *P. aeruginosa*

P. aeruginosa is intrinsically resistant to the majority of antimicrobial agents due to its selective ability to exclude various molecules from penetrating its outer membrane. The antimicrobial classes that remain active include some fluoroquinolones (e.g. ciprofloxacin and levofloxacin), aminoglycosides (e.g. gentamicin, tobramycin and amikacin), some beta-lactams (piperacillin–tazobactam, ceftazidime, cefepime, imipenem, doripenem and meropenem) and polymyxins (polymyxin B and colistin). Resistance of *P. aeruginosa* to these agents can be acquired through one or more of several mechanisms, including modified antimicrobial targets, active efflux, reduced permeability and degrading enzymes. Acquired resistance results from mutational changes in the bacterium and acquisition of plasmid mediated resistance genes. A growing concern is the emergence and spread of multidrug resistant *P. aeruginosa*, i.e. resistant to three or more classes of antimicrobials, in intensive care settings. Such resistance is due partly to the dissemination of carbapenemases in this species.

4.4 *Acinetobacter* spp.

The *Acinetobacter* genus consists of a large number of 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 a large number of environmental species with low pathogenicity). The correct identification of isolates at species level within *Acinetobacter* genus is challenging and is usually only possible with genotypic methods. Recently, mass spectrometry offers the

possibility of at least identifying isolates that belong to the *A. baumannii* group, which is by far the most clinically important group of species within this genus.

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. While many members of the *Acinetobacter* genus are considered ubiquitous in nature, this is not the case with the species that belong to the *A. baumannii* group. Carriage rates of species belonging to the *A. baumannii* group on the skin and in the faeces have been reported as very low.

Risk factors for infection with the *A. baumannii* group include advanced age, 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 antimicrobials. The risks for acquiring a multidrug-resistant strain of the *A. baumannii* group are similar, and also include prolonged mechanical ventilation, prolonged ICU or hospital stay, exposure to infected or colonized patients, increased frequency of interventions, increased disease severity and receipt of broad-spectrum antimicrobials, especially third-generation cephalosporins, fluoroquinolones and carbapenems.

4.4.1 Evolution of AMR in *Acinetobacter*

Acinetobacter spp. particularly those belonging in the *A. baumannii* group, are intrinsically resistant to most antimicrobial agents due to their selective ability to exclude various molecules from penetrating their outer membrane. The antimicrobial classes that remain active include some fluoroquinolones (e.g. ciprofloxacin and levofloxacin), aminoglycosides (e.g. gentamicin, tobramycin and amikacin), carbapenems (imipenem, doripenem and meropenem), polymyxins (polymyxins B and colistin) and, to some extent, sulbactam and tigecycline. Resistance of *Acinetobacter* spp. to these agents can be acquired through one or more of several mechanisms, including modified antimicrobial targets, active efflux, reduced permeability and degrading enzymes. Acquired resistance results from mutational changes in the bacterium and acquisition of plasmid mediated resistance genes. A growing concern is the emergence and spread of multidrug-resistant *Acinetobacter* spp., i.e. resistant to three or more classes of antimicrobials, in intensive care settings. Multidrug resistance in *Acinetobacter* spp. is frequently due to dissemination of carbapenemases.

4.5 *S. aureus*

S. aureus is a Gram-positive bacterium that can be a part of the normal microbiota on the skin and in the nose, but is another of the most important human pathogens. *S. aureus* can cause a variety of infections, most notably skin, soft tissue, bone and bloodstream infections. It is also the most common cause of postoperative wound infections. Some strains of *S. aureus* produce toxic factors that can cause a variety of specific symptoms, including toxic shock syndrome and food poisoning. Several successful *S. aureus* clones are responsible for the major part of the international spread and outbreaks in health care and community settings. A recent structured survey (6) showed that the most prevalent clones among methicillin-resistant *S. aureus* (MRSA) in EU countries are ST22 (EMRSA15), ST225 (New York/Japan), ST8 (US300), ST5 (New York/Japan), and ST8 (South German). Among methicillin-sensitive *S. aureus* (MSSA), the most prevalent clones are ST7, ST15, ST5, ST45 and ST8. The clonal structure of MRSA and MSSA in the CAESAR countries remains to be determined.

4.5.1 Evolution of AMR in *S. aureus*

When penicillin was first introduced in the 1940s, it was an effective treatment for *S. aureus* infections, but resistance had already developed within a few years of its introduction. This resistance was mediated

by the production of a beta-lactamase enzyme that inactivates drugs such as penicillin, ampicillin and amoxicillin. Consequently, beta-lactamase-stable drugs (e.g. methicillin and cloxacillin), as well as beta-lactamase inhibitors (e.g. clavulanic acid and sulbactam) that could be combined with the antibacterial drugs were developed. Strains of *S. aureus* resistant to these penicillinase-stable antibacterial drugs have acquired a novel gene (*mecA*, recently also *mecC*) that encodes a novel penicillin-binding protein (PBP); these strains are termed MRSA.

The first strains of MRSA emerged during the 1960s. Initially, MRSA was mainly a problem in hospital-acquired infections. Over the past decade, community-acquired MRSA has increased significantly in a number of countries. Fortunately, many of these community-acquired MRSA strains have so far retained susceptibility to a number of non-beta-lactam antibiotics, whereas most health care-associated MRSA infections are caused by difficult-to-treat multidrug-resistant strains. For the latter, the treatment of last resort has been glycopeptides such as vancomycin (since the 1950s) and teicoplanin, which can only be given by injection and also needs careful monitoring to avoid adverse side-effects. New treatment options for MRSA (but also associated with problematic side effects) have been developed more recently: linezolid (1970s) and daptomycin (1980s) are the most recently licensed antibacterial drug classes. In the last few years, some novel cephalosporins with activity against MRSA have also been developed (ceftaroline and ceftobiprole).

4.6 *S. pneumoniae*

S. pneumoniae is the leading cause worldwide of community-acquired pneumonia, which is among the leading causes of death of children under 5 years of age. Other diseases caused by *S. pneumoniae* include common, mild, self-limiting infections such as acute otitis media, but also extend to cases of invasive disease with high mortality such as meningitis. Among the bacterial causes of meningitis, *S. pneumoniae* is associated with the highest case-fatality rate and is the most likely to leave survivors with permanent residual symptoms. The clinical burden of pneumococcal infection is concentrated among the eldest and youngest sections of the population. According to one estimate, *S. pneumoniae* caused about 826 000 deaths (582 000–926 000) in children aged 1–59 months. For HIV-negative children, pneumococcal infection corresponds to 11% of all deaths in this age group (7). Pneumococci are commonly found in 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 day-care centres.

4.6.1 Evolution of AMR in *S. pneumoniae*

Resistance to beta-lactam antibacterial drugs in clinical isolates of *S. pneumoniae* occurs through the acquisition of mutations in the genes coding for the PBPs, which are essential components of the bacterial cell wall and the main target of beta-lactam antibiotics. The successive acquisition of multiple mutations in the different PBPs results in increasing minimum inhibitory concentrations (MICs) for penicillin and the other beta-lactam drugs. Different clinical breakpoints exist depending on the site of the *S. pneumoniae* infection (meningitis, bloodstream, lungs), as well as dosing regimens. Use of variable clinical breakpoints to interpret AST makes combining results and comparison of results difficult. If known, tables in this report will state which clinical breakpoints were used to interpret penicillin susceptibility at laboratory level.

4.7 *E. faecium* and *E. faecalis*

Enterococci belong to the normal bacterial microbiota of the gastrointestinal tract of both humans and other animals. Enterococci are usually low-pathogenic, but under certain circumstances can cause invasive disease. Recently, the recognition of high-risk clones suggests that some particular strains can act as true pathogens, and not only as opportunistic commensals. Enterococci can cause a variety

of infections, including endocarditis, bloodstream and urinary tract infections, and are associated with peritonitis and intra-abdominal abscesses. In the United States of America, enterococci cause 3–4 nosocomial bloodstream infections per 10 000 hospital discharges and contribute to patient mortality, as well as additional hospital stay.

The vast majority of clinical *Enterococcus* infections in humans are caused by *E. faecalis* and *E. faecium*. Epidemiological data collected over the last two decades have documented the emergence of enterococci as important nosocomial pathogens, 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*. The latter clones have even been isolated from farm animals. 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 treatment of human infections caused by *E. faecium* when resistance has emerged against penicillins. Besides the fact that infections caused by resistant strains are difficult to treat, enterococci are highly tenacious and thus easily disseminate in the hospital setting.

4.7.1 Evolution of AMR in Enterococci

Enterococci are intrinsically resistant to a broad range of antimicrobials including cephalosporins, sulphonamides and low concentrations of aminoglycosides. Patient safety in hospitals is challenged by the ability of enterococci to acquire additional resistance through the transfer of plasmids and transposons and recombination or mutation. By nature, enterococci have low susceptibility to many beta-lactam antibiotics as a consequence of their low-affinity PBPs. Resistance to aminopenicillin is currently rare in *E. faecalis*. Therefore, the first choice for treatment of infections caused by this microorganism is still an aminopenicillin such as ampicillin. In *E. faecium*, ampicillin resistance has increased significantly in recent years, not the least due to the wide dissemination of ampicillin-resistant strains belonging to the polyclonal subcluster CC17.

In addition to the intrinsic mechanism of low-level resistance to aminoglycosides, which causes a low uptake of the drug, enterococci have acquired genes conferring high-level resistance to aminoglycosides. The bifunctional APH(2'')/AAC(6') enzyme confers high-level resistance to all aminoglycosides except streptomycin and is now widespread across Europe. With high-level resistance, any synergistic effect between beta-lactams and glycopeptides is lost.

Glycopeptide resistance is due to the synthesis of modified cell wall precursors that show a decreased affinity for glycopeptides. Six phenotypes have been identified of which two have clinical relevance: VanA, with high-level resistance to vancomycin and a variable level of resistance to teicoplanin; and VanB, with a variable level of resistance in most cases to vancomycin only. The VanA and VanB phenotypes, mostly found among *E. faecalis* and *E. faecium*, may be transferred by mobile genetic elements.



CHAPTER

5

Reader's guide

5.1 Level of evidence

The goal of the AMR surveillance data collected and presented in this report is to provide a representative description of the antimicrobial susceptibility of common bacterial pathogens found in bloodstream infections to the main antimicrobial groups indicated for treatment of these infections. In other words, the aim is to provide the average susceptibility pattern of bacteria in patients presenting with a bloodstream infection before treatment is initiated (the target population). For a correct assessment of the magnitude and trends of AMR in the country and to allow comparison of results across countries, the data are required to be both reliable and representative.

The reliability and representativeness of data may be compromised at different points in the data generation process: from the selection of hospital laboratories that participate in the surveillance programme, to selection of patients for blood culturing in the clinic, to processing of samples in the laboratory, to aggregation and analysis of the data. In some countries, limiting conditions, outside the direct control of the national AMR surveillance system, may exist that reduce the reliability and representativeness of the data because they influence the selection of patients eligible for blood culturing or the quality of AST performed. Many different health care and public health professionals are involved in the many steps of the data generation process, requiring commitment and training at different levels to ensure good quality data.

Several sources of error and bias in AMR surveillance data are presented in Table 4 and discussed in detail in Annex 1. To guide the interpretation of the data, the authors together with the national focal points have judged the level of evidence for each country-specific data chapter.

At level A, data presented are judged to be representative for the target population, and AST results seem to be reliable. Data provide an adequate assessment of the magnitude and trends of AMR in the country.

At level B, indications that the data are not representative for the target population exist, but AST results seem to be reliable overall. Data provide an indication of resistance patterns present in clinical settings in the country, but the proportion resistance should be interpreted with care.

At level C, indications that the data are not representative for the target population exist, as well as doubts about the reliability of the AST data. Data do not provide an adequate assessment of the magnitude and trends of AMR in the country.

Importantly, the results with a low level of evidence are not necessarily wrong, but rather less representative for the target population due to errors in the data generation process. Though data may not yet be optimal, or issues leading to biased results may exist, the data are still presented. This is because having these surveillance data allows the critical appraisal of the data generation process, and provides an opportunity for constructive feedback to those involved in data generation and input for improvement of the process. Any suboptimal data presented in this report should be seen as a point of departure for further improvement.

Table 4. Sources of error and bias in AMR surveillance data

Type of error/bias		Mechanism	Solution
Random error	Sampling variation	Coincidence	Increase sample size
	Measurement variation	Test-to-test variation in application of laboratory procedures	Increase sample size Standardize procedures Continued training of laboratory staff Set up quality assurance programmes
BIAS DUE TO SAMPLING PROCEDURES			
	Selection of participating sites	Sampling special patient populations only, <i>e.g. tertiary hospitals, ICU, urban centres</i>	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, i.e. promote sampling of all cases with signs of bloodstream infection prior to treatment initiation
BIAS DUE TO LABORATORY PROCEDURES			
Systematic error	Laboratory standards	Use of non-uniform AST methods, <i>e.g. breakpoints from product inserts, out-of-date standards</i> Sequential testing, <i>e.g. test carbapenem only if resistance to third-generation cephalosporin</i>	Use national standards based on international standards for AST methodology (e.g. EUCAST) Test susceptibility to all indicator antimicrobials (uniform test panel) on all microorganisms
	Measurement error	Improper application of laboratory methods, <i>e.g. use of too large an inoculum</i> Inadequate laboratory materials, <i>e.g. use of expired or non-quality-controlled antimicrobial disks</i> Damaged, poorly calibrated equipment, <i>e.g. out-of-date firmware used with automated systems</i>	Laboratory staff training Laboratory quality assurance systems Confirmatory testing of highly resistant microorganisms Procurement of high-quality/quality-controlled materials
BIAS FROM DATA AGGREGATION AND ANALYSIS PROCEDURES			
		Include repeat isolates from individual patients Use of varying expert rules, <i>e.g. different rules for deriving resistance used in each laboratory</i>	Collect raw data Use standardized data aggregation and analysis methods

CHAPTER
6

Country-specific data

6.1 Belarus

6.1.1 Surveillance set-up

All results from routine AST of clinical bacteriology cultures of 10 (2012) and 15 (2014) clinical microbiology laboratories in Belarus are extracted with WHONET software and sent by email on a quarterly basis. Data are collected by the team from the National Reference Centre for AMR and the Republican Research and Practical Center for Epidemiology and Microbiology in Minsk. The data received by email are processed, its quality and consistency are checked, problems are detected and feedback is provided to the laboratories to improve data quality in the future. Confirmatory testing of highly resistant microorganisms and unexpected phenotypes are performed before results are included in the final dataset, but results are not always available due to problems in isolates selection and transferral to the National Reference Centre for AMR. A subset of AST results, containing all first isolates from blood and CSF cultures yielding organisms specified by CAESAR, for the period 1 January–31 December 2012 were provided to CAESAR.

The 10 participating (out of approximately 30) laboratories provide diagnostic support for approximately 70% of hospitals, including the Republican Research and Practical Center for Epidemiology and Microbiology. The participating laboratories are geographically spread out, but some large Belarusian urban centres and regions are underrepresented because they use laboratory software incompatible with WHONET. The largest part of the data (approximately 70%) represents the laboratory of the Minsk City Centre of Hygiene and Epidemiology, which provides diagnostic support for the majority of Minsk clinics (about 30% of the Belarusian population).

Antimicrobial susceptibility is mostly tested using a disk diffusion method and automated system. One laboratory is able to use gradient tests, though only in some combinations of microorganisms/antimicrobials or for confirmation purposes. If highly resistant microorganisms or exceptional phenotypes are found, results are confirmed by retesting using all available methods. All laboratories apply quality management systems and are audited on a regular basis by the Belarusian State Committee for Standardization and Methodology. Since 2013, eight laboratories from all regions of Belarus take part in the international (CAESAR, UK NEQAS) EQA exercise. Also since 2013, four national laboratories, including the National Reference Centre for AMR, take part in the National Institute for Communicable Diseases/WHO globally coordinated EQA programme for the WHO Global Invasive Bacterial Vaccine Preventable Diseases (IBVPD) Laboratory Network.

Laboratories are required to follow national guidelines on bacteriologic methods published in 2009. For AST methods and interpretation, Belarus has adopted CLSI 2004 methodology as the national standard. According to national clinical guidelines, blood cultures should be taken from all patients with suspected bloodstream infections (sepsis) presenting in hospital, and CSF cultures are taken from patients suspected of having meningitis. The number of haemocultures, especially from patients with pneumonia, is still very low. Bacteriology cultures and AST is financed by the national budget.

6.1.2 Results

Table 5 shows the patient characteristics of 386 isolates from Belarus in 2012, by pathogen. In Enterobacteriaceae, resistance was over 50% for all tested antimicrobials except for carbapenems (Table 6). Carbapenem resistance was 0% for *E. coli* and 3% for *K. pneumoniae*. Also for *P. aeruginosa* and *Acinetobacter* spp., overall resistance was higher than 50% (Table 7). More than 80% of isolates in both

of the latter species were carbapenem resistant; whereas ceftazidime resistance in *P. aeruginosa* was lower (59%). Importantly, only a low number of *P. aeruginosa* isolates were available, and the proportion of resistance should, therefore, be interpreted with caution. Thirty-five percent of *S. aureus* isolates were MRSA (Table 8). There was only one *S. pneumoniae* isolate. Therefore, resistance in *S. pneumoniae* was not calculated. Seventy-six percent of *E. faecalis* isolates were resistant to aminopenicillins (Table 9). In *E. faecium*, vancomycin and linezolid resistance were both 3%.

Table 5. Patient characteristics of 386 isolates from Belarus in 2012, by pathogen

Pathogen	Total isolates (N)	Isolate source (%)		Sex (%)			Age category (years) (%)					Hospital department (%)		
		Blood	CSF	Male	Female	Unknown	0–4	5–19	20–64	≥ 65	Unknown	ICU	Non-ICU	Unknown
<i>E. coli</i>	33	100	0	6	45	49	9	0	33	15	43	0	0	100
<i>K. pneumoniae</i>	77	95	5	36	29	35	4	0	39	21	36	1	0	99
<i>P. aeruginosa</i>	18	100	0	44	11	45	6	6	33	6	49	0	0	100
<i>Acinetobacter</i> spp.	128	95	5	45	37	18	13	5	51	19	12	0	1	99
<i>S. aureus</i>	38	100	0	37	37	26	0	5	55	24	16	0	3	97
<i>S. pneumoniae</i>	1	100	0	0	100	0	0	100	0	0	0	0	100	0
<i>E. faecalis</i>	51	100	0	53	18	29	4	4	45	27	20	0	0	100
<i>E. faecium</i>	40	100	0	25	30	45	13	5	33	15	34	0	0	100

Table 6. Resistance levels for *E. coli* and *K. pneumoniae* among blood and CSF isolates in Belarus

Antibiotic class	<i>E. coli</i>		<i>K. pneumoniae</i>	
	N	Resistance (%)	N	Resistance (%)
Aminopenicillins (R) ^a	33	94	NA	NA
3rd-generation cephalosporins (R) ^b	30	87	76	92
3rd-generation cephalosporins (I+R) ^b	30	87	76	92
Aminoglycosides (R) ^c	33	58	74	89
Fluoroquinolones (R) ^d	32	75	77	84
Fluoroquinolones (I+R) ^d	32	75	77	87
Carbapenems (R) ^e	25*	0*	65	3
Carbapenems (I+R) ^e	25*	0*	65	3

NA: not applicable.

* A low number of isolates were tested (N < 30), and the percentage resistance should be interpreted with caution.

^a The aminopenicillins group consists of amoxicillin and ampicillin.

^b The third-generation cephalosporin group consists of cefotaxime, ceftriaxone and ceftazidime.

^c The aminoglycoside group consists of amikacin, gentamicin and tobramycin.

^d The fluoroquinolone group consists of ciprofloxacin, ofloxacin and levofloxacin.

^e The carbapenem group consists of imipenem and meropenem.

Table 7. Resistance levels for *P. aeruginosa* and *Acinetobacter* spp. among blood and CSF isolates in Belarus

Antibiotic class	<i>P. aeruginosa</i>		<i>Acinetobacter</i> spp.	
	N	Resistance (%)	N	Resistance (%)
Aminoglycosides (R) ^a	15*	87*	105	55
Amikacin (R)	12*	75*	0*	-
Fluoroquinolones (R) ^b	18*	89*	124	86
Piperacillin/Piperacillin-tazobactam (R)	18*	78*	NA	NA
Ceftazidime (R)	17*	59*	NA	NA
Carbapenems (R) ^c	15*	87*	118	81
Carbapenems (I+R) ^c	15*	87*	118	81

NA: not applicable; -: resistance not calculated.

* A low number of isolates were tested (N < 30), and the percentage resistance should be interpreted with caution.

^a The aminoglycoside group consists of gentamicin and tobramycin.

^b The fluoroquinolone group consists of ciprofloxacin and levofloxacin.

^c The carbapenem group consists of imipenem and meropenem.

Table 8. Resistance levels for *S. aureus* among blood and CSF isolates in Belarus

Antibiotic class	<i>S. aureus</i>	
	N	Resistance (%)
MRSA ^a	34	35
Fluoroquinolones (R) ^b	33	21
Rifampicin (R)	32	16
Linezolid (R)	30	0

^a MRSA is calculated as resistance against one or more out of methicillin, oxacillin, flucloxacillin, cloxacillin, dicloxacillin or cefoxitin.

^b The fluoroquinolone group consists of ciprofloxacin, ofloxacin, levofloxacin and norfloxacin.

Table 9. Resistance levels for *E. faecium* and *E. faecalis* among blood and CSF isolates in Belarus

Antibiotic class	<i>E. faecalis</i>		<i>E. faecium</i>	
	N	Resistance (%)	N	Resistance (%)
Aminopenicillins (I+R) ^a	50	76	38	95
High-level gentamicin (R)	5*	0*	9*	0*
Vancomycin (R)	51	0	39	3
Teicoplanin (R)	7*	0*	6*	0*
Linezolid (I+R)	49	0	37	3

* A low number of isolates were tested (N < 30), and the percentage resistance should be interpreted with caution.

^a The aminopenicillins group consists of amoxicillin and ampicillin.

6.1.3 Discussion

In 2012, AST results of 386 isolates from blood or CSF were submitted by the AMR surveillance network of Belarus. Compared to other species, few *E. coli* isolates were isolated. In general, high resistance levels were found. The combination of a low number of isolates, a skewed distribution of pathogens and high resistance levels indicates selective sampling of patients, e.g., severely ill patients with a history of hospitalization and antimicrobial treatment, patients from wards with high selective pressure of antimicrobials (e.g. ICUs) or patients who failed to respond to empiric antimicrobial treatment. Therefore, the reported resistance levels should be interpreted with caution and are not necessarily generalizable to all patients presenting with a bloodstream infection in Belarus.

Nevertheless, the data indicate that resistance to third-generation cephalosporins, likely mediated by ESBLs, is common in the sampled patients. The low proportion of carbapenem resistance detected could be related to the use of old CLSI breakpoints, which have low sensitivity for detection of low-level carbapenem resistance. The high aminopenicillin resistance in *E. faecalis* may reflect problems with species identification (inclusion of *E. faecium*, which normally is resistant to aminopenicillins). The level of MRSA is similar to countries close to Belarus (5). For *S. pneumoniae*, no interpretation can be carried out due to the lack of data. The high levels of resistance in *P. aeruginosa* and *Acinetobacter* spp. are concerning and possibly due to expansion of resistant clones in the health care setting.

Suggestions to improve the representativeness of the surveillance data include increasing the number of isolates by including more hospital types and departments from different geographical regions in Belarus, and promoting the sampling of all cases with signs of bloodstream infection prior to treatment initiation. In addition, large proportions of unknown patient characteristics, such as sex, age category and hospital department, indicate a need to put more emphasis on completing the isolate record forms.

The authors regard the data available from Belarus as **Level B data**. There are indications that the data are not representative for the target population, but AST results seem to be reliable overall. Data provide an indication of resistance patterns present in clinical settings in the country, but the proportion resistance should be interpreted with care.

6.2 Serbia

6.2.1 Surveillance set-up

All results from routine AST of the first isolates from blood and CSF cultures yielding organisms specified by CAESAR are collected twice a year (for the six-month period 1 January–30 June and 1 July–31 December) from the laboratory network of 14 microbiology laboratories in Serbia.

Data are collected by the National Reference Laboratory for AMR – the Center for Microbiology of the Institute for Public Health of Vojvodina – in Novi Sad, Serbia. As data come in, its quality and consistency are checked, errors are fed back to the laboratories and corrected where applicable, and then the data are uploaded into the national WHONET database.

The 14 participating laboratories provide diagnostic support for general hospitals, academic and top clinical hospitals, including the largest clinical centres in the country. They are geographically spread and cover about 50% of the population.

Antimicrobial susceptibility is mostly tested using the disk diffusion method; some laboratories use a combination of an automated system and disk diffusion, and gradient tests when needed, according to CLSI. A switch to EUCAST was planned before the end of 2015.

Several laboratories are accredited according to the International Organization for Standardization (ISO)/International Electrotechnical Commission 17025, and some according to ISO 9001 and ISO 14001 standards. All 14 laboratories have internal quality control schemes and took part in the national and international (UK NEQAS) EQA. There is no regular national EQA programme. Reference laboratories are nominated by the Ministry of Health but funding is insufficient, no additional staff could be allocated, and sending of the reports and bacterial strains to reference laboratories is not regulated, but done on a voluntary basis. There are no published national guidelines on bacteriologic methods for testing antimicrobial susceptibility.

Blood cultures are taken from all patients with suspected bloodstream infections (sepsis), and CSF cultures are taken from patients suspected of having meningitis. Bacteriology cultures are reimbursed through the National Health Insurance Fund.

6.2.2 Results

Table 10 shows the patient characteristics of 1465 isolates from Serbia in 2013, by pathogen. In *E. coli*, resistance was over 25% for all tested antimicrobials except for carbapenems (3%, Table 11). Resistance in *K. pneumoniae* ranged from 36% for carbapenems to 88% for third-generation cephalosporins. Resistance levels between 19% and 51% were found in *P. aeruginosa*, and overall resistance in *Acinetobacter* spp. was higher than 90% (Table 12). Forty-two percent of *S. aureus* isolates were MRSA (Table 13). In *S. pneumoniae*, 19% of isolates were resistant to penicillins (Table 14). Forty-one percent of *E. faecalis* isolates were aminopenicillin resistant (Table 15). Vancomycin resistance was 9% in *E. faecalis* and 75% in *E. faecium*. In *E. faecium*, 2% linezolid resistance was found.

Table 10. Patient characteristics of 1465 isolates from Serbia in 2013, by pathogen

Pathogen	Total isolates (N)	Isolate source (%)		Sex (%)			Age category (years) (%)				Hospital department (%)		
		Blood	CSF	Male	Female	Unknown	0–4	5–19	20–64	≥ 65	ICU	Non-ICU	Unknown
<i>E. coli</i>	199	98	2	45	54	1	12	11	47	30	9	86	5
<i>K. pneumoniae</i>	307	97	3	60	34	6	17	8	47	28	31	65	4
<i>P. aeruginosa</i>	109	95	5	57	40	3	9	4	53	34	27	70	3
<i>Acinetobacter</i> spp.	392	91	9	61	37	2	14	4	57	25	38	60	2
<i>S. aureus</i>	270	100	0	59	38	3	17	8	50	25	14	79	7
<i>S. pneumoniae</i>	42	55	45	50	48	2	10	2	69	19	50	45	5
<i>E. faecalis</i>	78	100	0	67	27	6	13	3	53	32	24	73	3
<i>E. faecium</i>	68	100	0	79	19	2	18	6	44	32	21	78	1

6.2.3 Discussion

In 2013, AST results of 1465 isolates from blood or CSF were submitted by the Serbian AMR surveillance network. Compared with other species, there was a small number of *E. coli* isolates. In general, high resistance levels were found. The combination of a relatively low number of isolates, skewed distribution of pathogens and high resistance levels is an indication of selective sampling of isolates (e.g. in more severely ill

Table 11. Resistance levels for *E. coli* and *K. pneumoniae* among blood and CSF isolates in Serbia

Antibiotic class	<i>E. coli</i>		<i>K. pneumoniae</i>	
	N	Resistance (%)	N	Resistance (%)
Aminopenicillins (R) ^a	191	69	NA	NA
3rd-generation cephalosporins (R) ^b	195	32	304	88
3rd-generation cephalosporins (I+R) ^b	195	33	304	88
Aminoglycosides (R) ^c	198	30	307	78
Fluoroquinolones (R) ^d	190	27	293	67
Fluoroquinolones (I+R) ^d	190	29	293	73
Carbapenems (R) ^e	199	3	306	36
Carbapenems (I+R) ^e	199	3	306	38

NA: not applicable.

^a The aminopenicillins group consists of amoxicillin and ampicillin.

^b The third-generation cephalosporin group consists of cefotaxime, ceftriaxone and ceftazidime.

^c The aminoglycoside group consists of amikacin, gentamicin and tobramycin.

^d The fluoroquinolone group consists of ciprofloxacin, ofloxacin and levofloxacin.

^e The carbapenem group consists of imipenem and meropenem.

Table 12. Resistance levels for *P. aeruginosa* and *Acinetobacter* spp. among blood and CSF isolates in Serbia

Antibiotic class	<i>P. aeruginosa</i>		<i>Acinetobacter</i> spp.	
	N	Resistance (%)	N	Resistance (%)
Aminoglycosides (R) ^a	99	51	369	91
Amikacin (R)	109	40	0	-
Fluoroquinolones (R) ^b	106	47	386	91
Piperacillin/Piperacillin-tazobactam (R)	106	19	NA	NA
Ceftazidime (R)	108	44	NA	NA
Carbapenems (R) ^c	109	47	392	93
Carbapenems (I+R) ^c	109	48	392	93

NA: not applicable; -: resistance not calculated.

^a The aminoglycoside group consists of gentamicin and tobramycin.

^b The fluoroquinolone group consists of ciprofloxacin and levofloxacin.

^c The carbapenem group consists of imipenem and meropenem.

patients or patients who fail to respond to antimicrobial treatment). Therefore, the reported resistance levels should be interpreted with caution, and are not necessarily generalizable to all patients presenting with a bloodstream infection in Serbia.

Table 13. Resistance levels for *S. aureus* among blood and CSF isolates in Serbia

Antibiotic class	<i>S. aureus</i>	
	N	Resistance (%)
MRSA (R) ^a	270	42
Fluoroquinolones (R) ^b	249	33
Rifampicin (R)	213	10
Linezolid (R)	161	0

^a MRSA is calculated as resistance against one or more out of methicillin, oxacillin, flucloxacillin, cloxacillin, dicloxacillin or ceftiofur.

^b The fluoroquinolone group consists of ciprofloxacin, ofloxacin, levofloxacin and norfloxacin.

Table 14. Resistance levels for *S. pneumoniae* among blood and CSF isolates in Serbia

Antibiotic class	<i>S. pneumoniae</i>	
	N	Resistance (%)
Penicillins (R) ^a	42	19
Penicillins (I+R) ^a	42	21
Macrolides (R) ^b	41	29
Macrolides (I+R) ^b	41	29
3rd-generation cephalosporins (R) ^c	33	0
3rd-generation cephalosporins (I+R) ^c	33	0
Fluoroquinolones (R) ^d	11*	0*
Moxifloxacin (R)	0	-

–: resistance not calculated.

* A low number of isolates were tested (N < 30), and the percentage resistance should be interpreted with caution.

^a The penicillins group consists of penicillin and oxacillin, clinical breakpoint unknown.

^b The macrolide group consists of erythromycin, clarithromycin and azithromycin.

^c The third-generation cephalosporin group consists of cefotaxime and ceftriaxone.

^d The fluoroquinolone group consists of ciprofloxacin, ofloxacin, levofloxacin and norfloxacin.

Table 15. Resistance levels for *E. faecium* and *E. faecalis* among blood and CSF isolates in Serbia

Antibiotic class	<i>E. faecalis</i>		<i>E. faecium</i>	
	N	Resistance (%)	N	Resistance (%)
Aminopenicillins (I+R) ^a	78	41	66	94
High-level gentamicin (R)	77	68	66	95
Vancomycin (R)	78	9	68	75
Teicoplanin (R)	54	7	62	79
Linezolid (I+R)	67	0	61	2

^a The aminopenicillins group consists of amoxicillin and ampicillin.

Nevertheless, a high level of carbapenem resistance was seen in *K. pneumoniae* and a very high level of third-generation cephalosporin resistance was seen in both *K. pneumoniae* and *E. coli* in the specific patient population sampled. Additionally, resistance to multiple antimicrobials was high in *Pseudomonas* isolates from these patients. Resistance proportions in *Acinetobacter* isolates were above 90% for all antimicrobial groups tested. The aminopenicillin resistance in *E. faecalis* was high. This result may reflect problems with species identification (inclusion of *E. faecium*, which is normally resistant to aminopenicillins), rather than true high resistance in *E. faecalis*. The high occurrence of high-level gentamicin resistance is unexpected and may be related to the potency of the disks that were used for susceptibility testing. Furthermore, 2% linezolid resistance in *E. faecium* is higher than expected. This finding could reflect laboratory errors and unconfirmed test results. The high resistance levels in the nosocomial pathogens *P. aeruginosa* and *Acinetobacter* spp. are concerning, and may reflect dissemination in the health care setting.

The representativeness of the surveillance data in Serbia can be increased by increasing the diversity of hospitals and departments from different regions, and decreasing selective sampling by promoting sampling of all cases with signs of bloodstream infection prior to treatment initiation.

The authors regard the data available from Serbia as **Level B data**. There are indications that the data are not representative for the target population, but AST results seem to be reliable overall. Data provides an indication of resistance patterns present in clinical settings in the country, but the proportion resistance should be interpreted with care.

6.3 Switzerland

6.3.1 Surveillance set-up

The Swiss Centre for Antibiotic Resistance was set up in 2004 in the frame of a national research programme. It is run by the Institute for Infectious Diseases, University of Bern and financed by the Swiss Federal Office of Public Health, the Swiss Conference of the Cantonal Ministers of Public Health and the University of Bern.

Twenty laboratories send all results from routine AST of all clinical bacteriology cultures on a regular basis (weekly or monthly) to a central database. There is no systematic confirmation of the delivered results. A subset of AST results, containing all first isolates from blood and CSF cultures yielding organisms specified by CAESAR, for the period 1 January–31 December 2013 were provided to CAESAR.

The 20 participating laboratories represent about 70% of all hospitalized patients and one third of all ambulatory practitioners. The laboratories are geographically spread over all Swiss regions, and include university and general hospital laboratories, as well as private laboratories.

There are no national AST guidelines. Most Swiss laboratories changed from CLSI to EUCAST guidelines between 2011 and 2013. Most laboratories use automated systems; unusual AST results are confirmed locally, and invasive *S. pneumoniae* isolates are sent to a national reference centre for AST and serotyping. All laboratories are participating in at least one national or international external quality programme. Therefore, Switzerland decided not participating in the CAESAR EQA exercise. Blood cultures are taken from all patients with suspected bloodstream infections presenting in hospital, and CSF cultures are taken from patients suspected of having meningitis. Bacteriological cultures are reimbursed through the universal health insurance scheme.

6.3.2 Results

Table 16 shows the patient characteristics of 7945 isolates from Switzerland in 2013, by pathogen. In Enterobacteriaceae, 7% was resistant to third-generation cephalosporins, and 0% (*E. coli*) and 1% (*K. pneumoniae*) were resistant to carbapenems (Table 17). Resistance levels in *P. aeruginosa* and

Acinetobacter spp. ranged from 1% (amikacin in *P. aeruginosa*) to 11% (carbapenems in *Acinetobacter* spp., Table 18). Five percent of *S. aureus* isolates were MRSA (Table 19). In *S. pneumoniae*, 2% of isolates were resistant to penicillins (Table 20). Resistance of *E. faecium* to aminopenicillins was 81% (Table 21). Vancomycin resistance was 0% in both species.

Table 16. Patient characteristics of 7945 isolates from Switzerland in 2013, by pathogen

Pathogen	Total isolates (N)	Isolate source (%)		Sex (%)		Age category (years) (%)				Hospital department (%)	
		Blood	CSF	Male	Female	0–4	5–19	20–64	≥ 65	ICU	Non-ICU
<i>E. coli</i>	3 994	100	0	47	53	2	1	30	67	4	96
<i>K. pneumoniae</i>	708	99	1	60	40	3	1	33	63	7	93
<i>P. aeruginosa</i>	375	100	0	69	31	4	1	34	61	11	89
<i>Acinetobacter</i> spp.	54	98	2	59	41	7	0	37	56	11	89
<i>S. aureus</i>	1 413	100	0	65	35	3	3	33	61	8	92
<i>S. pneumoniae</i>	615	97	3	53	47	3	4	37	56	4	96
<i>E. faecalis</i>	462	100	0	67	33	5	1	23	71	9	91
<i>E. faecium</i>	324	100	0	62	38	2	1	38	59	15	85

Table 17. Resistance levels for *E. coli* and *K. pneumoniae* among blood and CSF isolates in Switzerland

Antibiotic class	<i>E. coli</i>		<i>K. pneumoniae</i>	
	N	Resistance (%)	N	Resistance (%)
Aminopenicillins (R) ^a	3 687	49	NA	NA
3rd-generation cephalosporins (R) ^b	3 983	7	707	7
3rd-generation cephalosporins (I+R) ^b	3 983	8	707	8
Aminoglycosides (R) ^c	3 991	8	705	5
Fluoroquinolones (R) ^d	3 992	16	706	6
Fluoroquinolones (I+R) ^d	3 992	17	706	7
Carbapenems (R) ^e	3 990	0	706	1
Carbapenems (I+R) ^e	3 990	0	706	1

NA: not applicable.

^a The aminopenicillins group consists of amoxicillin and ampicillin.

^b The third-generation cephalosporin group consists of cefotaxime, ceftriaxone and ceftazidime.

^c The aminoglycoside group consists of amikacin, gentamicin and tobramycin.

^d The fluoroquinolone group consists of ciprofloxacin, ofloxacin and levofloxacin.

^e The carbapenem group consists of imipenem and meropenem.

Table 18. Resistance levels for *P. aeruginosa* and *Acinetobacter* spp. among blood and CSF isolates in Switzerland

Antibiotic class	<i>P. aeruginosa</i>		<i>Acinetobacter</i> spp.	
	N	Resistance (%)	N	Resistance (%)
Aminoglycosides (R) ^a	375	5	52	10
Amikacin (R)	352	1	0	-
Fluoroquinolones (R) ^b	374	10	53	11
Piperacillin/Piperacillin-tazobactam (R)	366	7	NA	NA
Ceftazidime (R)	357	6	NA	NA
Carbapenems (R) ^c	372	9	53	11
Carbapenems (I+R) ^c	372	10	53	11

NA: not applicable; -: resistance not calculated.

^a The aminoglycoside group consists of gentamicin and tobramycin.

^b The fluoroquinolone group consists of ciprofloxacin and levofloxacin.

^c The carbapenem group consists of imipenem and meropenem.

Table 19. Resistance levels for *S. aureus* among blood and CSF isolates in Switzerland

Antibiotic class	<i>S. aureus</i>	
	N	Resistance (%)
MRSA (R) ^a	1 408	5
Fluoroquinolones (R) ^b	1 412	8
Rifampicin (R)	1 398	0
Linezolid (R)	731	0

^a MRSA is calculated as resistance against one or more out of methicillin, oxacillin, flucloxacillin, cloxacillin, dicloxacillin or cefoxitin.

^b The fluoroquinolone group consists of ciprofloxacin, ofloxacin, levofloxacin and norfloxacin.

6.3.3 Discussion

In 2013, AST results of 7945 isolates from blood or CSF were submitted by the AMR surveillance network of Switzerland. There is no indication of selective sampling of patients. Therefore, the reported resistance levels are expected to be representative of patients presenting with a bloodstream infection in Switzerland.

For all pathogens, resistance levels are similar to countries close to Switzerland (5). In particular, a low proportion of resistance in Gram-negative bacteria was seen. For *S. pneumoniae*, susceptibility testing for third-generation cephalosporins was not performed for all isolates. However, because resistance levels are low, it is not expected that this has biased the results much. Carbapenem resistance in *Acinetobacter* spp. was relatively high, which probably indicates dissemination of carbapenemase-producing strains in the health care setting.

The authors regard the data available from Switzerland as **Level A data**. Data presented are judged to be representative for the target population, and AST results seem to be reliable. Data provide an adequate assessment of the magnitude and trends of AMR in the country.

Table 20. Resistance levels for *S. pneumoniae* among blood and CSF isolates in Switzerland

Antibiotic class	<i>S. pneumoniae</i>	
	N	Resistance (%)
Penicillins (R) ^a	545	2
Penicillins (I+R) ^a	545	5
Macrolides (R) ^b	529	11
Macrolides (I+R) ^b	529	11
3rd-generation cephalosporins (R) ^c	400	0
3rd-generation cephalosporins (I+R) ^c	400	2
Fluoroquinolones (R) ^d	458	3
Moxifloxacin (R)	0	-

–: resistance not calculated.

^a The penicillins group consists of penicillin and oxacillin, meningitis breakpoint (R > 0.06 mg/L) was used.

^b The macrolide group consists of erythromycin, clarithromycin and azithromycin.

^c The third-generation cephalosporin group consists of cefotaxime and ceftriaxone.

^d The fluoroquinolone group consists of ciprofloxacin, ofloxacin, levofloxacin and norfloxacin.

Table 21. Resistance levels for *E. faecium* and *E. faecalis* among blood and CSF isolates in Switzerland

Antibiotic class	<i>E. faecalis</i>		<i>E. faecium</i>	
	N	Resistance (%)	N	Resistance (%)
Aminopenicillins (I+R) ^a	410	1	282	81
High-level gentamicin (R)	141	13	108	37
Vancomycin (R)	431	0	290	0
Teicoplanin (R)	0	-	0	-
Linezolid (I+R)	308	1	217	0

–: resistance not calculated.

^a The aminopenicillins group consists of amoxicillin and ampicillin.

6.4 The former Yugoslav Republic of Macedonia

6.4.1 Surveillance set-up

All results from routine AST of clinical bacteriology cultures are collected on paper on a monthly basis from six microbiology laboratories in the former Yugoslav Republic of Macedonia. Data are collected by the CAESAR national data team independent from the national AMR surveillance system managed by the Institute for Public Health. As data comes in, its quality and consistency are checked, and errors are fed back to the laboratories and corrected where applicable. Confirmatory testing of highly resistant microorganisms is required before results are included in the final dataset. A subset of AST results,

containing all first isolates from blood and CSF cultures yielding organisms specified by CAESAR, for the period 1 January 2013–31 December 2013 were provided to CAESAR.

The six participating (out of 23 public and private) laboratories provide diagnostic support for approximately 70% of hospitals, including academic, clinical and general hospitals. The six laboratories are geographically spread out in the western part of the country. The eastern side, which has one large clinical centre and a few general hospitals, is not covered.

Antimicrobial susceptibility is mostly tested using automated systems, but laboratories are also using disk diffusion tests in their routine work. One laboratory is using gradient tests for MICs. If highly resistant microorganisms or exceptional phenotypes are found, results are confirmed by gradient tests. No laboratories (for clinical microbiology) in the country are accredited by a national accreditation institute yet, but all took part in the international (UK NEQAS) external quality control programmes (2013 and 2014).

Laboratories are required to follow national guidelines on bacteriologic methods for testing special resistance. For methods and interpretation of AST, laboratories still use the CLSI system, but are in the process of adopting EUCAST methodology as the national standard. EUCAST guidelines were translated and distributed to all laboratories, workshops for implementation of EUCAST methodology were performed and the laboratories are in the process of procurement of antimicrobial disks according to EUCAST standards. According to national clinical guidelines, blood cultures are taken from all patients with suspected bloodstream infections (sepsis) presenting in hospital, and CSF cultures are taken from patients suspected of having meningitis. Bacteriology cultures are reimbursed through the national health insurance fund. The number of haemocultures is low due to lack of financial resources.

6.4.2 Results

Table 22 shows the patient characteristics of 189 isolates in 2013, by pathogen. In Enterobacteriaceae, resistance was over 50% for all tested antimicrobials except for fluoroquinolones in *K. pneumoniae* (26%) and carbapenems (0%) in both species (Table 23). In *Acinetobacter* spp., overall resistance was higher than 40% (Table 24); in particular, carbapenem resistance was 71%. Forty-one percent of *S. aureus* isolates were MRSA (Table 25). Importantly, only a low number of *P. aeruginosa*, *Acinetobacter* spp., *S. pneumoniae*, and *Enterococcus* spp. isolates were available; therefore, the proportion of resistance should be interpreted with caution (Tables 26–27).

6.4.3 Discussion

In 2013, AST results of 189 isolates from blood or CSF were submitted from the AMR surveillance network of the former Yugoslav Republic of Macedonia. Compared with other species, few *E. coli* were isolated. In general, high resistance levels were found. The combination of these low numbers of isolates, skewed distribution of pathogens and high resistance levels, is an indication of selective sampling of isolates; e.g. severely ill patients with a history of hospitalization and antimicrobial treatment, patients from wards with high selective pressure of antimicrobials (e.g. ICUs) or patients who failed to respond to empiric antimicrobial treatment. Therefore, the reported resistance levels should be interpreted with caution, and are not necessarily generalizable to all patients presenting with a bloodstream infection in the former Yugoslav Republic of Macedonia.

There was an unexpected low number of *S. pneumoniae* isolates. This is because in case of pneumonia, it is more common to take samples from other body sides (e.g. sputum) than blood or CSF. Carbapenem resistance in *K. pneumoniae* was isolated from sterile isolates. In the former Yugoslav Republic of Macedonia, carbapenem resistant *K. pneumoniae* is found in samples from other body sites (e.g. urine), but not (yet) in blood or CSF (Dr Golubinka Bosevska, Institute of Public Health, personal communication, 26 November 2014). Although few isolates of *P. aeruginosa* and *Acinetobacter* spp. were tested, the resistance levels in these nosocomial pathogens are of concern, and may reflect dissemination in the health care setting.

Suggestions to improve the representativeness of the surveillance data include increasing the number of participating hospitals types and departments from different geographical regions, and promoting sampling of all cases with signs of bloodstream infection prior to treatment initiation. In addition, more emphasis should be put on completing the isolate record forms to decrease the proportions of unknown patient characteristics, such as sex, age category and hospital department.

Table 22. Patient characteristics of 189 isolates from the former Yugoslav Republic of Macedonia in 2013, by pathogen

Pathogen	Total isolates (N)	Isolate source (%)		Sex (%)			Age category (years) (%)					Hospital department (%)		
		Blood	CSF	Male	Female	Unknown	0–4	5–19	20–64	≥ 65	Unknown	ICU	Non-ICU	Unknown
<i>E. coli</i>	50	98	2	52	42	6	12	4	50	16	18	41	40	46
<i>K. pneumoniae</i>	35	100	0	46	17	37	63	0	29	6	2	49	37	14
<i>P. aeruginosa</i>	5	100	0	60	40	0	20	0	80	0	0	20	60	20
<i>Acinetobacter</i> spp.	17	100	0	65	35	0	18	0	53	12	17	47	35	18
<i>S. aureus</i>	55	100	0	58	36	6	13	2	40	31	14	4	40	56
<i>S. pneumoniae</i>	5	60	40	60	40	0	20	20	60	0	0	0	60	40
<i>E. faecalis</i>	18	100	0	61	33	6	6	0	50	33	11	11	50	39
<i>E. faecium</i>	4	100	0	100	0	0	0	0	75	25	0	0	50	50

Table 23. Resistance levels for *E. coli* and *K. pneumoniae* among blood and CSF isolates in the former Yugoslav Republic of Macedonia

Antibiotic class	<i>E. coli</i>		<i>K. pneumoniae</i>	
	N	Resistance (%)	N	Resistance (%)
Aminopenicillins (R) ^a	10*	70*	NA	NA
3rd-generation cephalosporins (R) ^b	49	59	35	89
3rd-generation cephalosporins (I+R) ^b	49	59	35	89
Aminoglycosides (R) ^c	50	50	35	83
Fluoroquinolones (R) ^d	50	52	35	26
Fluoroquinolones (I+R) ^d	50	52	35	26
Carbapenems (R) ^e	48	0	35	0
Carbapenems (I+R) ^e	48	0	35	0

NA: not applicable.

* A low number of isolates were tested (N < 30), and the percentage resistance should be interpreted with caution.

^a The aminopenicillins group consists of amoxicillin and ampicillin.

^b The third-generation cephalosporin group consists of cefotaxime, ceftriaxone and ceftazidime.

^c The aminoglycoside group consists of amikacin, gentamicin and tobramycin.

^d The fluoroquinolone group consists of ciprofloxacin, ofloxacin and levofloxacin.

^e The carbapenem group consists of imipenem and meropenem.

The authors regard the data available from the former Yugoslav Republic of Macedonia as **Level B data**. There are indications that the data are not representative for the target population, but AST results seem to be reliable overall. Data provides an indication of resistance patterns present in clinical settings in the country, but the proportion resistance should be interpreted with care.

Table 24. Resistance levels for *P. aeruginosa* and *Acinetobacter* spp. among blood and CSF isolates in the former Yugoslav Republic of Macedonia

Antibiotic class	<i>P. aeruginosa</i>		<i>Acinetobacter</i> spp.	
	N	Resistance (%)	N	Resistance (%)
Aminoglycosides (R) ^a	4*	25*	17*	41*
Amikacin (R)	5*	0*	0	-
Fluoroquinolones (R) ^b	5*	20*	17*	88*
Piperacillin/Piperacillin-tazobactam (R)	5*	20*	NA	NA
Ceftazidime (R)	3*	0*	NA	NA
Carbapenems (R) ^c	5*	20*	17*	71*
Carbapenems (I+R) ^c	5*	40*	17*	71*

NA: not applicable; -:resistance not calculated.

* A low number of isolates were tested (N < 30), and the percentage resistance should be interpreted with caution.

^a The aminoglycoside group consists of gentamicin and tobramycin.

^b The fluoroquinolone group consists of ciprofloxacin and levofloxacin.

^c The carbapenem group consists of imipenem and meropenem.

Table 25. Resistance levels for *S. aureus* among blood and CSF isolates in the former Yugoslav Republic of Macedonia

Antibiotic class	<i>S. aureus</i>	
	N	Resistance (%)
MRSA (R) ^a	54	41
Fluoroquinolones (R) ^b	53	9
Rifampicin (R)	47	11
Linezolid (R)	53	0

^a MRSA is calculated as resistance against one or more out of methicillin, oxacillin, flucloxacillin, cloxacillin, dicloxacillin or cefoxitin.

^b The fluoroquinolone group consists of ciprofloxacin, ofloxacin, levofloxacin and norfloxacin.

Table 26. Resistance levels for *S. pneumoniae* among blood and CSF isolates in the former Yugoslav Republic of Macedonia

Antibiotic class	<i>S. pneumoniae</i>	
	N	Resistance (%)
Penicillins (R) ^a	5*	40*
Penicillins (I+R) ^a	5*	40*
Macrolides (R) ^b	5*	60*
Macrolides (I+R) ^b	5*	60*
3rd-generation cephalosporins (R) ^c	5*	0*
3rd-generation cephalosporins (I+R) ^c	5*	20*
Fluoroquinolones (R) ^d	4*	0*
Moxifloxacin (R)	4*	0*

* A low number of isolates were tested (N < 30), and the percentage resistance should be interpreted with caution.

^a The penicillins group consists of penicillin and oxacillin, clinical breakpoint unknown.

^b The macrolide group consists of erythromycin, clarithromycin and azithromycin.

^c The third-generation cephalosporin group consists of cefotaxime and ceftriaxone.

^d The fluoroquinolone group consists of ciprofloxacin, ofloxacin, levofloxacin and norfloxacin.

Table 27. Resistance levels for *E. faecium* and *E. faecalis* among blood and CSF isolates in the former Yugoslav Republic of Macedonia

Antibiotic class	<i>E. faecalis</i>		<i>E. faecium</i>	
	N	Resistance (%)	N	Resistance (%)
Aminopenicillins (I+R) ^a	15*	33*	4*	100*
High-level gentamicin (R)	16*	69*	4*	100*
Vancomycin (R)	18*	0*	4*	75*
Teicoplanin (R)	15*	0*	4*	50*
Linezolid (I+R)	17*	0*	4*	25*

* A low number of isolates were tested (N < 30), and the percentage resistance should be interpreted with caution.

^a The aminopenicillins group consists of amoxicillin and ampicillin.

6.5 Turkey

6.5.1 Surveillance set-up

The Turkish National AMR Surveillance System was established in 2011. Data are collected by the National Reference Laboratory for AMR at the Public Health Institution of Turkey of the Ministry of Health. AST results isolated from blood and CSF cultures are collected into a standard database in six-month intervals from participating laboratories. As data come in, its quality and consistency are checked; errors are fed back to the laboratories and corrected where applicable. After these processes, the data are converted into CAESAR data

format via Backlink in WHONET. A subset of AST results, containing all first isolates from blood and CSF cultures yielding organisms specified by CAESAR, for the period 1 January–31 December 2013 were provided to CAESAR.

The participating laboratories were selected from different geographical regions of the country to reflect the distribution of the population. Of a total of 77 laboratories, 35 are clinical microbiology laboratories of university hospitals, and 42 are clinical microbiology laboratories of state hospitals.

Antimicrobial susceptibility is mostly tested using automated systems (57 laboratories), and 20 laboratories use disk diffusion and gradient tests. Some laboratories use a combination of automated systems and disk diffusion/gradient tests when needed, according to CLSI.

All laboratories have implemented internal quality control. The national external quality control programme is applied to participating laboratories once a year by the Public Health Institution of Turkey, since 2011. Laboratories participating in CAESAR also participate in an international EQA (UK NEQAS). Turkey has published national guidelines on bacteriologic methods for testing antimicrobial susceptibility, which were updated in 2014. The methodology of the AMR surveillance system is compatible with CAESAR methodology. Laboratories currently use CLSI standards, but in late 2015, the new EUCAST based standard will be implemented. EUCAST documents were translated into Turkish in 2014.

According to national clinical guidelines, blood cultures are taken from all patients with suspected bloodstream infections presenting in hospital, and CSF cultures are taken from patients suspected of having meningitis. If unusual resistance is detected, isolates should be sent to the reference centre for confirmation. Bacteriology cultures are reimbursed through the National Health Insurance Fund.

6.5.2 Results

Table 28 shows the patient characteristics of 10 377 isolates from Turkey in 2013, by pathogen. *Acinetobacter* species were only collected in the National AMR Surveillance System starting in 2014; therefore, no data were available on *Acinetobacter* spp. For *E. coli*, resistance was over 20% for all tested antimicrobials except for carbapenems (4%, Table 29). Carbapenem resistance in *K. pneumoniae* was 11%,

Table 28. Patient characteristics of 10 377 isolates from Turkey in 2013, by pathogen

Pathogen	Total isolates (N)	Isolate source (%)		Sex (%)		Age category (years) (%)					Hospital department (%)		
		Blood	CSF	Male	Female	0–4	5–19	20–64	≥ 65	Unknown	ICU	Non-ICU	Unknown
<i>E. coli</i>	3 286	99	1	51	49	7	7	38	34	14	19	77	4
<i>K. pneumoniae</i>	1 635	98	2	58	42	13	4	35	32	16	42	54	4
<i>P. aeruginosa</i>	1 123	97	3	59	41	8	7	33	36	16	45	53	2
<i>Acinetobacter</i> spp.	0	-	-	-	-	-	-	-	-	-	-	-	-
<i>S. aureus</i>	2 133	100	0	66	34	7	5	37	35	16	29	67	4
<i>S. pneumoniae</i>	147	94	6	65	35	6	7	36	25	26	18	76	6
<i>E. faecalis</i>	1 136	100	0	56	44	6	3	27	43	21	49	47	4
<i>E. faecium</i>	917	100	0	52	48	7	4	26	40	23	47	49	4

Table 29. Resistance levels for *E. coli* and *K. pneumoniae* among blood and CSF isolates in Turkey

Antibiotic class	<i>E. coli</i>		<i>K. pneumoniae</i>	
	N	Resistance (%)	N	Resistance (%)
Aminopenicillins (R) ^a	1 424	67	NA	NA
3rd-generation cephalosporins (R) ^b	2 223	44	1 168	56
3rd-generation cephalosporins (I+R) ^b	2 223	45	1 168	59
Aminoglycosides (R) ^c	2 401	22	1 280	30
Fluoroquinolones (R) ^d	2 020	41	1 171	34
Fluoroquinolones (I+R) ^d	2 020	42	1 171	39
Carbapenems (R) ^e	2 046	4	1 083	11
Carbapenems (I+R) ^e	2 046	5	1 083	15

NA: not applicable.

^a The aminopenicillins group consists of amoxicillin and ampicillin.

^b The third-generation cephalosporin group consists of cefotaxime, ceftriaxone and ceftazidime.

^c The aminoglycoside group consists of amikacin, gentamicin and tobramycin.

^d The fluoroquinolone group consists of ciprofloxacin, ofloxacin and levofloxacin.

^e The carbapenem group consists of imipenem and meropenem.

Table 30. Resistance levels for *P. aeruginosa* and *Acinetobacter* spp. among blood and CSF isolates in Turkey

Antibiotic class	<i>P. aeruginosa</i>		<i>Acinetobacter</i> spp.	
	N	Resistance (%)	N	Resistance (%)
Aminoglycosides (R) ^a	771	19	0	-
Amikacin (R)	656	11	0	-
Fluoroquinolones (R) ^b	845	22	0	-
Piperacillin/Piperacillin-tazobactam (R)	795	27	NA	NA
Ceftazidime (R)	785	26	NA	NA
Carbapenems (R) ^c	821	33	0	-
Carbapenems (I+R) ^c	821	36	0	-

NA: not applicable; -: resistance not calculated

^a The aminoglycoside group consists of gentamicin and tobramycin.

^b The fluoroquinolone group consists of ciprofloxacin and levofloxacin.

^c The carbapenem group consists of imipenem and meropenem.

with resistance to other antimicrobials at 30% or higher. Thirty-three percent resistance to carbapenems was found in *P. aeruginosa* (Table 30). Of all *S. aureus* isolates tested, 26% were MRSA, and 2% were reported to be linezolid resistant (Table 31). In *S. pneumoniae*, 54% penicillin resistance was found (Table 32). Resistance to vancomycin was 1% in *E. faecalis* and 23% in *E. faecium* (Table 33). In these respective organisms, 2% and 4% linezolid resistance was reported.

Table 31. Resistance levels for *S. aureus* among blood and CSF isolates in Turkey

Antibiotic class	<i>S. aureus</i>	
	N	Resistance (%)
MRSA (R) ^a	1 136	26
Fluoroquinolones (R) ^b	1 059	22
Rifampicin (R)	809	17
Linezolid (R)	1 125	2

^a MRSA is calculated as resistance against one or more out of methicillin, oxacillin, flucloxacillin, cloxacillin, dicloxacillin or ceftioxin.
^b The fluoroquinolone group consists of ciprofloxacin, ofloxacin, levofloxacin and norfloxacin.

Table 32. Resistance levels for *S. pneumoniae* among blood and CSF isolates in Turkey

Antibiotic class	<i>S. pneumoniae</i>	
	N	Resistance (%)
Penicillins (R) ^a	82	54
Penicillins (I+R) ^a	82	55
Macrolides (R) ^b	106	42
Macrolides (I+R) ^b	106	42
3rd-generation cephalosporins (R) ^c	58	5
3rd-generation cephalosporins (I+R) ^c	58	19
Fluoroquinolones (R) ^d	79	0
Moxifloxacin (R)	0	-

–: resistance not calculated.

^a The penicillins group consists of penicillin and oxacillin, meningitis breakpoint (R > 0.06 mg/L) was used.

^b The macrolide group consists of erythromycin, clarithromycin and azithromycin.

^c The third-generation cephalosporin group consists of cefotaxime and ceftriaxone.

^d The fluoroquinolone group consists of ciprofloxacin, ofloxacin, levofloxacin and norfloxacin.

Table 33. Resistance levels for *E. faecium* and *E. faecalis* among blood and CSF isolates in Turkey

Antibiotic class	<i>E. faecalis</i>		<i>E. faecium</i>	
	N	Resistance (%)	N	Resistance (%)
Aminopenicillins (I+R) ^a	788	5	638	99
High-level gentamicin (R)	561	25	434	49
Vancomycin (R)	829	1	636	23
Teicoplanin (R)	538	0	411	18
Linezolid (I+R)	709	2	568	4

^a The aminopenicillins group consists of amoxicillin and ampicillin.

6.5.3 Discussion

In 2013, AST results of 10 377 isolates from blood or CSF were submitted by the Turkish National AMR Surveillance System. The low number of *S. pneumoniae* isolates was unexpected. This may indicate infrequent routine blood culturing of severe pneumonia cases, and selective sampling may, therefore, be in part responsible for the high resistance levels found for *S. pneumoniae*. For the other pathogens, no indication for selective sampling of patients was found. The reported resistance levels are likely to be representative of patients presenting with a bloodstream infection in Turkey.

The high level of carbapenem resistance that was found in *E. coli*, is a result of a large outbreak and subsequent spread of OXA-48 strains in Turkey (8). Also in *P. aeruginosa*, carbapenem resistance was high. Multidrug-resistant *P. aeruginosa* is a known problem in Turkey (9). Linezolid resistance in *S. aureus* and enterococci is rare worldwide. Therefore, the observed resistance levels are likely to reflect laboratory errors and unconfirmed test results.

To decrease the proportions of unknown patient characteristics, such as age category and hospital department, more emphasis should be put on completing the isolate record forms.

The authors regard the data available from Turkey as **Level A data**. The data presented are judged to be representative for the target population, and AST results seem to be reliable. Data provide an adequate assessment of the magnitude and trends of AMR in the country.



CHAPTER

7

CAESAR EQA

7.1 Introduction

A panel of six lyophilized isolates were prepared and found fully compliant in quality control testing by UK NEQAS, and results were confirmed in two expert reference laboratories. The panel included an *A. baumannii* group, *S. pneumoniae*, *S. aureus*, *E. coli*, *K. pneumoniae* and *P. aeruginosa*. The EQA panels were dispatched on 4 November 2013 to a total of 131 participants in eight of the 10 countries or areas participating in the CAESAR network. Participants were requested to return results within five weeks.

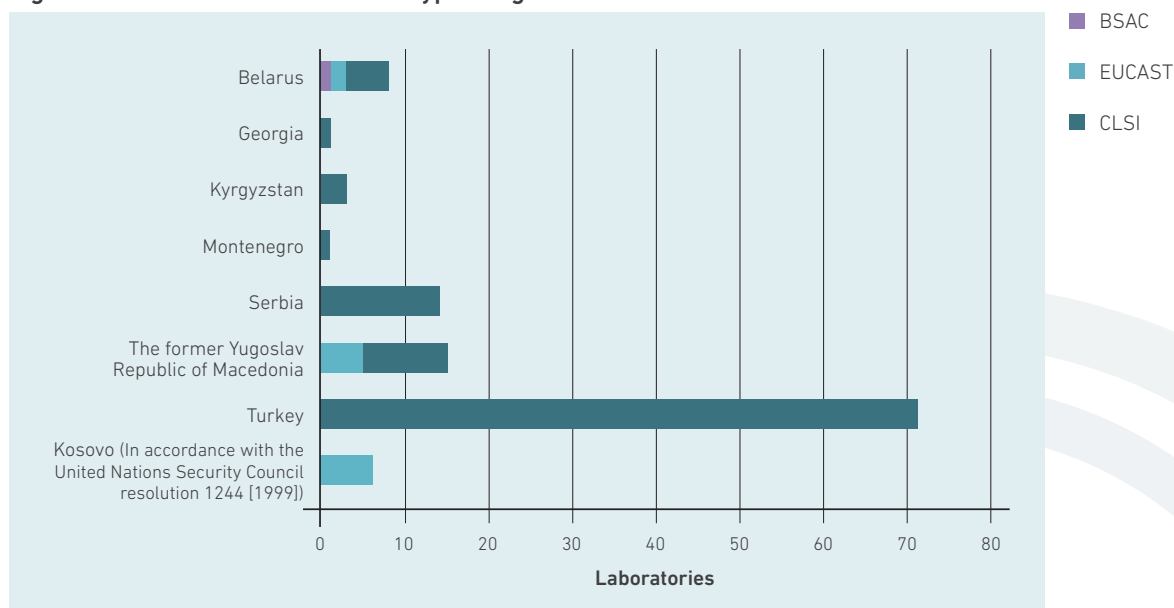
Results were returned from eight countries/areas by 120/131 (92%) participants: 8/8 laboratories from the Belarus network, 1/1 from Georgia, 6/7 from the Kosovo² network, 3/3 from the Kyrgyzstan network, 1/1 from Montenegro, 14/14 from the Serbia network, 15/16 from the former Yugoslav Republic of Macedonia network and 72/78 from the Turkey network. These are the national surveillance networks that send their data to the international CAESAR network.

7.2 Results

7.2.1 Methods and guidelines used

A breakdown of the methods and guidelines used by participants examining the EQA specimens is presented in Fig. 2. All participants followed international guidelines: CLSI (88%) and EUCAST or EUCAST-related (14%). A breakdown of methods used revealed that 49% of laboratories used an automated instrument, 47% used a disk diffusion susceptibility method and of the remaining participants, two performed MICs, one used gradient test and two participants did not specify a method.

Fig. 2. Number of laboratories and types of guidelines used



BSAC: British Society for Antimicrobial Chemotherapy.

² In accordance with United Nations Security Council resolution 1244 (1999).

7.2.2 Antimicrobial susceptibility results

Participants' results were collated, analysed and presented in individual laboratory reports, which were subsequently uploaded onto the secure UK NEQAS website (10). The reports display the individual laboratory's results and the overall results for all laboratories so that laboratories can make suitable comparisons. Participants can access their reports at any time, as well as download a printed copy.

Overall, performance was generally very good and consistent with that seen in previous EQA surveys among participants in EU countries. Problems, where experienced, were related to borderline susceptibility. EQA is a valuable tool in the quality assurance of AST, and indicates the validity of comparing collated data between laboratories in resistance surveillance studies. The different isolates are described in more detail on the next pages, and the results per country are in Tables 34–39.

The susceptibility interpretation of the pathogens isolated against the antimicrobial agents tested were defined as intermediate (I), resistant (R) or sensitive (S).

Specimen 1950 was an *A. baumannii* that was susceptible to all reference agents tested (Table 34).

Table 34. *A. baumannii* group (specimen 1950): MIC and intended results reported by the reference laboratories and the percentage of laboratories giving the correct result per country, territory or area

Agent	MIC range (mg/L) reference laboratory		Intended interpretation	Percentage of laboratories giving the correct result								
	From	To		EUCAST/CLSI	Belarus	Georgia	Kyrgyzstan	Montenegro	MKD	Serbia	Turkey	Kosovo*
Amikacin	2	2	S/S	100	100	100	100	100	100	100	100	100
Ciprofloxacin	0.25	0.5	S/S	100	100	100	100	93	100	99	100	100
Colistin	0.25	0.5	S/S	100	100	NA	NA	100	100	100	100	100
Doripenem	0.12	0.12	S/-	67	NA	NA	NA	0	100	75	NA	NA
Gentamicin	0.5	0.5	S/S	100	100	100	100	100	100	100	100	100
Imipenem	0.12	0.25	S/S	100	0	100	100	93	100	100	83	83
Meropenem	0.25	0.25	S/S	100	0	100	100	100	100	99	100	100
Tobramycin	1	1	S/S	100	NA	100	NA	100	100	100	100	100

MKD: The former Yugoslav Republic of Macedonia. MKD is an abbreviation of ISO.

NA: not assigned.

* (in accordance with United Nations Security Council resolution 1244 (1999))

Specimen 1951 was an *E. coli* resistant to ampicillin/amoxicillin. Susceptibility to amoxicillin-clavulanic acid (co-amoxiclav) was borderline susceptible (MIC 8 mg/L) by EUCAST and CLSI breakpoints (Table 35).

Table 35. *E. coli* (specimen 1951): MIC and intended results reported by the reference laboratories and the percentage of laboratories giving the correct result per country, territory or area

Agent	MIC range (mg/L) reference laboratory		Intended interpretation	Percentage of laboratories giving the correct result							
	From	To		EUCAST/CLSI	Belarus	Georgia	Kyrgyzstan	Montenegro	MKD	Serbia	Turkey
Amikacin	1	1	S/S	100	100	100	100	93	100	100	100
Amoxicillin	≥ 128	≥ 128	R/R	100	NA	100	NA	100	100	91	100
Amoxicillin-clavulanic acid	8	8	S/S	100	100	100	100	100	100	97	100
Ampicillin	≥ 128	≥ 128	R/R	63	0	67	100	93	75	89	20
Cefotaxime	0.015	0.06	S/S	100	100	100	100	92	100	97	100
Ceftazidime	0.06	0.12	S/S	88	100	100	100	80	100	94	83
Ceftriaxone	0.06	0.06	S/S	100	100	100	100	100	100	97	83
Ciprofloxacin	0.015	0.015	S/S	100	100	100	100	100	100	99	83
Doripenem	0.015	0.12	S/S	100	NA	NA	NA	NA	100	93	NA
Ertapenem	≥ 0.004	0.008	S/S	100	100	NA	100	50	100	100	100
Gentamicin	≥ 0.06	0.5	S/S	100	100	100	100	93	100	100	100
Imipenem	0.12	0.12	S/S	100	100	100	100	100	100	100	83
Levofloxacin	-	-	S*	100	100	100	NA	100	100	100	100
Meropenem	0.015	0.015	S/S	100	100	100	100	100	100	100	100
Ofloxacin	-	-	S*	100	NA	100	100	100	100	100	100
Piperacillin-tazobactam	1	1	S/S	88	100	100	100	85	93	97	100
Tobramycin	1	1	S/S	100	NA	100	NA	93	100	91	100
ESBL confirmation	-	-	Negative	100	NA	NA	100	90	100	98	100

MKD: The former Yugoslav Republic of Macedonia. MKD is an abbreviation of ISO.

NA: not assigned.

* Results based on participants' consensus, because no reference laboratory results are available.

[#] (in accordance with United Nations Security Council resolution 1244 (1999))

Specimen 1952 was a *K. pneumoniae* that produces an OXA-48 carbapenemase. Isolates producing OXA-48 enzymes frequently show borderline resistance to carbapenems, and may appear fully susceptible to cephalosporins. In reference MIC tests, this isolate was resistant to ertapenem by both EUCAST and CLSI breakpoints (ertapenem MIC 8–64 mg/L), and intermediate to both imipenem and meropenem by EUCAST breakpoints but resistant to both agents by CLSI breakpoints (imipenem and meropenem MICs both 4 mg/L). Doripenem reference MICs were 1–4 mg/L, which straddles the EUCAST susceptible/intermediate breakpoint, and ranges from susceptible to resistant with CLSI breakpoints (Table 36).

Table 36. *K. pneumoniae* (specimen 1952): MIC and intended results reported by the reference laboratories and the percentage of laboratories giving the correct result per country, territory or area

Agent	MIC range (mg/L) reference laboratory		Intended interpretation	Percentage of laboratories giving the correct result							
	From	To		EUCAST/CLSI	Belarus	Georgia	Kyrgyzstan	Montenegro	MKD	Serbia	Turkey
Amikacin	0.5	2	S/S	100	100	100	100	100	100	100	100
Amoxicillin	≥ 128	≥ 128	R/R	100	NA	100	NA	100	100	100	100
Amoxicillin-clavulanic acid	≥ 128	≥ 128	R/R	100	100	100	100	100	100	95	100
Ampicillin	≥ 128	≥ 128	R/R	100	100	67	100	100	100	100	100
Cefotaxime	2	2	I/I	38	0	33	100	23	36	22	17
Ceftazidime	1	1	S/S	75	100	100	100	80	100	96	40
Ceftriaxone	1	1	S/S	71	100	100	0	83	69	94	67
Ciprofloxacin	0.03	0.03	S/S	100	100	100	100	100	93	97	100
Doripenem	-	-	S/I, S/R*	NA	NA	NA	NA	NA	NA	NA	NA
Ertapenem	8	64	R/R	100	100	NA	100	50	92	96	100
Gentamicin	0.25	0.5	S/S	100	100	100	100	100	100	100	83
Imipenem	4	4	I/R	38	0	0	0	33	7	21	40
Levofloxacin	-	-	S*	100	100	100	NA	100	100	100	100
Meropenem	4	4	I/R	0	0	0	0	17	15	21	50
Ofloxacin	-	-	S*	100	NA	100	100	100	100	100	100
Piperacillin-tazobactam	≥ 128	≥ 128	R/R	100	100	100	100	92	100	96	100
Tobramycin	0.25	0.25	S/S	100	NA	100	NA	100	100	97	75
ESBL confirmation	-	-	Negative	80	NA	NA	100	100	100	92	33

MKD: The former Yugoslav Republic of Macedonia. MKD is an abbreviation of ISO. NA: not assigned.

* Results based on participants' consensus, because no reference laboratory results are available.

(in accordance with United Nations Security Council resolution 1244 (1999))

Specimen 1953 was a MRSA.

Table 37. *S. aureus* (specimen 1953): MIC and intended results reported by the reference laboratories and the percentage of laboratories giving the correct result per country, territory or area

Agent	MIC range (mg/L) reference laboratory		Intended interpretation	Percentage of laboratories giving the correct result							
	From	To		EUCAST/CLSI	Belarus	Georgia	Kyrgyzstan	Montenegro	MKD	Serbia	Turkey
Cefoxitin	≥ 128	≥ 128	R/R	100	100	100	100	93	100	98	50
Ciprofloxacin	≥ 128	≥ 128	R/R	100	100	100	100	93	100	100	100
Clindamycin	≥ 128	≥ 128	R/R	88	100	100	100	100	86	87	100
Erythromycin	≥ 128	≥ 128	R/R	100	NA	100	100	100	100	99	100
Fusidic acid	0.12	0.25	S/-	100	NA	NA	100	80	100	98	83
Gentamicin	0.25	0.5	S/S	100	100	100	100	100	93	97	83
Oxacillin	≥ 128	≥ 128	R/R	100	100	100	100	100	100	100	100
Penicillin	≥ 128	≥ 128	R/R	100	100	100	100	100	100	100	100
Rifampicin	≤ 0.004	0.008	S/S	100	100	NA	100	86	73	65	75
Teicoplanin	0.5	1	S/S	100	NA	NA	NA	100	100	98	50
Tetracycline	0.25	0.5	S/S	100	100	100	100	100	100	100	83
Vancomycin	0.5	1	S/S	100	100	100	100	100	100	99	80

MKD: The former Yugoslav Republic of Macedonia. MKD is an abbreviation of ISO.

NA: not assigned.

[#] (in accordance with United Nations Security Council resolution 1244 (1999))

Specimen 1954 was a multiresistant *S. pneumoniae*. The organism showed resistance to all reference agents tested except levofloxacin and moxifloxacin (Table 38). For *S. pneumoniae* with no mechanism of resistance to penicillin, MICs are ≤ 0.06 mg/L. For isolates with higher MICs the interpretation of susceptibility to penicillin depends on the site of infection. Patients with pneumonia caused by strains with intermediate susceptibility (MIC 0.12–2 mg/L) are, depending on the dosage, treatable with penicillin, ampicillin or amoxicillin. Hence, such strains may be reported susceptible if from pneumonia. Patients with meningitis caused by strains with penicillin MIC > 0.06 mg/L are unlikely to respond to therapy, and such strains should be reported as resistant in this situation. Both EUCAST and CLSI guidelines include options for reporting susceptibility depending on the site of infection. Although EUCAST breakpoints for isolates other than meningitis are $S \leq 0.06$ mg/L and $R > 2$ mg/L, notes indicate that isolates with MICs above 0.06 mg/L are susceptible with the higher doses used to treat pneumonia, the breakpoint depending on the dose. This distribution uses the lowest high dose quoted by EUCAST to define breakpoints for pneumonia as $S \leq 0.5$ mg/L and $R > 2$ mg/L. However, the penicillin MIC of 4–8 mg/L for this isolate is resistant by EUCAST breakpoints irrespective of the type of infection, and borderline intermediate/resistant for pneumonia by CLSI breakpoints.

Table 38. *S. pneumoniae* (specimen 1954): MIC and intended results reported by the reference laboratories and the percentage of laboratories giving the correct result per country, territory or area

Agent	MIC range (mg/L) reference laboratory		Intended interpretation	Percentage of laboratories giving the correct result								
	From	To		EUCAST/CLSI	Belarus	Georgia	Kyrgyzstan	Montenegro	MKD	Serbia	Turkey	Kosovo [#]
Cefotaxim	8	16		NA	NA	NA	NA	NA	NA	NA	NA	NA
Meningitis			R/R	100	NA	67	100	83	100	93	67	
Pneumonia			R/R	100	NA	67	100	83	67	84	67	
Ceftriaxon	8	8		NA	NA	NA	NA	NA	NA	NA	NA	NA
Meningitis			R/R	88	NA	33	100	75	100	97	67	
Pneumonia			R/R	88	NA	33	100	75	62	82	100	
Clindamycin	> 256	> 256	R/-	88	100	100	100	100	100	98	100	
Erythromycin	≥ 128	≥ 128	R	100	100	100	100	100	100	100	100	
Levofloxacin	1	1	S	100	100	100	NA	91	100	97	NA	
Moxifloxacin	0.06	0.12	S	100	100	100	NA	100	100	100	100	
Norfloxacin	-	-	S/-*	100	NA	100	100	100	100	92	100	
Oxacillin screen	-	-	R	100	100	100	100	100	100	98	100	
Penicillin	4	8	-	NA	NA	NA	NA	NA	NA	NA	NA	NA
Meningitis			R/R	100	NA	NA	100	70	25	56	100	
Pneumonia			R/IR	100	NA	NA	100	100	100	92	67	

MKD: The former Yugoslav Republic of Macedonia. MKD is an abbreviation of ISO. NA: not assigned.
[#] (in accordance with United Nations Security Council resolution 1244 (1999))

Specimen 1956 was a *P. aeruginosa* with carbapenem resistance typical of isolates with upregulated efflux and OprD porin loss. In addition, the organism produces a VEB (Vietnamese extended-spectrum beta-lactamase) ESBL. The organism showed resistance to all reference agents tested except polymyxins and piperacillin-tazobactam. The piperacillin-tazobactam MIC (16 mg/L) is borderline susceptible (Table 39).

Table 39. *P. aeruginosa* (specimen 1956): MIC and intended results reported by the reference laboratories and the overall concordance of the participating laboratories

Agent	MIC range (mg/L) reference laboratory		Intended interpretation	Percentage of laboratories giving the correct result							
	From	To		EUCAST/CLSI	Belarus	Georgia	Kyrgyzstan	Montenegro	MKD	Serbia	Turkey
Amikacin	64	≥ 128	R/R	100	100	0	100	100	100	94	67
Cefepime	-	-	R*	100	100	100	100	100	100	100	100
Ceftazidime	≥ 128	≥ 128	R/R	100	100	100	100	100	100	99	100
Ciprofloxacin	32	32	-/(R*)	100	100	100	100	100	100	100	100
Doripenem	≥ 16	≥ 16	-/(R*)	100	NA	NA	NA	NA	100	81	NA
Gentamicin	≥ 128	≥ 128	R/R	100	100	100	100	100	100	100	100
Imipenem	16	16	R/R	100	100	67	0	93	100	96	100
Levofloxacin	-	-	R*	100	100	100	NA	100	100	99	100
Meropenem	16	32	R/R	100	100	33	100	100	93	97	83
Piperacillin-tazobactam	16	16	S/S	71	100	100	100	77	71	57	100
Tobramycin	≥ 128	≥ 128	R/R	100	NA	100	NA	100	100	100	100

MKD: The former Yugoslav Republic of Macedonia. MKD is an abbreviation of ISO.

NA: not assigned; -/-: no official intended interpretation from either of the reference laboratories.

* Results based on participants' consensus, because no reference laboratory results are available.

[#] (in accordance with United Nations Security Council resolution 1244 (1999))



CHAPTER

8

Concluding remarks

The aim of this report is not only to present the first data collected through the CAESAR network, but also to provide guidance to countries building or strengthening their national AMR surveillance and to stimulate the sharing of data internationally. This is a central element in the global approach to combat AMR as laid out in the draft global action plan on AMR put forward for adoption during the Sixty-eighth World Health Assembly in May 2015 (11).

When comparing the CAESAR results with those reported by EARS-Net, overall the resistance proportions reported by the CAESAR countries fall within the same range of resistance proportions reported by the southern and eastern European countries. The resistance levels reported by Switzerland are similar to those reported by its surrounding countries. The national surveillance network, the Swiss Centre for Antibiotic Resistance, provides representative data for patients presenting with a bloodstream infection. Also the data presented from Turkey, with the exception of *S. pneumoniae* resistance, are considered to be representative. The high level of carbapenem resistance found in *E. coli* is a result of a large outbreak and subsequent spread of OXA-48 strains in Turkey. From Belarus, Serbia and the former Yugoslav Republic of Macedonia, especially higher proportions of resistance were reported for *Acinetobacter* spp. and *P. aeruginosa*. Though this may reflect dissemination in health care settings, these data should be interpreted with caution, due to indications that the data are not representative for the population, as described in the country specific chapters.

Even though there are concerns described in the report regarding potential laboratory errors that might have influenced the results in some cases, the performance of the laboratories participating in the CAESAR EQA was generally very good and consistent with that seen in previous EQA surveys among laboratories in the EU countries.

One of the main areas to improve surveillance data relate to representativeness of the data. Issues such as selective sampling, both in terms of geographical representation, as well as in the severity of illness of sampled patients, need to be addressed to be able to generalize the results from the surveillance system. This will be an important focus of the continued efforts of CAESAR to support countries in joining the network and improving national surveillance.

Even though, for some of the countries the data displayed in this report should be interpreted with caution, the levels of resistance reported confirm the need for action, and emphasize the importance of good clinical practice in limiting the further development of AMR. Using surveillance data to increase awareness among policy-makers and the public and to provide treatment guidance to physicians is essential in fighting AMR.

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ANNEX
1

Sources of error and bias in AMR surveillance data

When interpreting results from surveillance or any other form of research, one should always assess whether the result reflects the truth. Every measurement of reality has a risk of deviating from the truth, either due to random or systematic error. Random deviation is due to chance variation, which occurs during sampling or measurement. Systematic deviations are due to systematic errors in collection, processing and analysis of the data. Systematic deviation is also called bias. In particular, systematic deviation may occur due to choices made when taking patient samples (e.g. sampling bias), when processing samples in the laboratory (e.g. measurement error) or when aggregating data for analysis (e.g. duplicate isolates).

Random error will always occur, and the amount of error can be reduced to a certain extent by the investigators. Systematic error, on the other hand, can be reduced significantly by the investigators by paying attention to details of and improving the data generation process.

Random error

Sampling variation

Random error may occur due to chance whenever a sample of individuals is taken from a population. For example, if counting the number of patients presenting with signs of a bloodstream infection from whom a blood culture is obtained each week over the period of four consecutive weeks, a different number will have been submitted each week (e.g. 9, 13, 10 and 11) during the first, second, third and fourth week, respectively. This is consistent with a true average of 11 blood cultures per week, but the observed number of blood cultures varies per week due to chance. Random variation may result in either over- or underestimation of a resistance proportion. The amount of deviation from reality expected due to random error, or the statistical precision of a measurement, is a function of sample size. The smaller the sample size, the larger the potential deviation is from reality; the larger the sample size, the smaller the potential variation.

Measurement variation

Random error also occurs whenever measurements are taken and will result from slight variations in the way measurement procedures are applied from measurement to measurement. For example, the concentration of an inoculum that is plated out will vary every time. Random variation will result in either over- or underestimation of a resistance proportion. In general, these deviations will be a mix of over- or underestimation and will cancel each other out when results are combined. Again, sample size will reduce the effect of random highs and lows. The amount of error in automated measuring systems will generally be small and within acceptable bounds. With human procedures, the amount of error depends on the experience of the person doing the test and the care taken during the measurement procedures. Standardizing procedures, training of laboratory staff and quality assurance will work towards minimizing random measurement variation.

Systematic error

Bias due to sampling procedures

Selection of participating sites

In order to obtain a nationally representative assessment of AMR, the hospital laboratories selected for participation in the national surveillance should be from different geographical and climatic regions, include both rural and urban areas, and provide samples from different patient populations (hospital types/departments). Sampling only special populations will only allow the generalization of results to that specific population, but not necessarily to the overall patient population.

Selection of patients

When surveillance is based on routine diagnostic testing, as in this report, data should be interpreted with extra caution. Because data used in passive surveillance is not generated with surveillance as the primary objective, rather with patient care as the aim, these data are inherently biased towards more ill patients, patients in whom treatment is problematic or patients with high suspicion of having resistant infections; i.e., clinical predictions are taken along in the decision whether or not to test. In active surveillance, on the other hand, clear case definitions are generally used to identify patients that need to be sampled; i.e., specific efforts are made to attain a representative sample of the target population.

In order to obtain results that are representative of the target population, one should make certain that all patients fitting the case definition are sampled; in the case of CAESAR, all patients presenting with signs of a bloodstream infection (signs of systemic inflammatory response syndrome) should be sampled. Including only special patient categories (only ICU, tertiary care institutions), or patients with chronic/recurring infection, relapses or treatment failure will overestimate the resistance proportion, because these patients were subjected to selective pressure of antimicrobials. Utilization of microbiologic diagnostics is subject to financial and logistical constraints outside the control of the surveillance system. For example, few blood cultures may be taken in routine clinical care if the cost of bacteriologic sampling is not reimbursed through health insurance, or if physicians are not used to sampling every patient due to low laboratory capacity. Also sampling of patients may be frequent after antimicrobial therapy has already been started, or after failure of self-treatment if sales of over-the-counter antimicrobials are legal.

When samples are collected may also influence the resistance proportions found. Any seasonal variation will be overcome by sampling throughout the year. Ad hoc or convenience sampling for a limited time period, in particular during outbreaks, will bias results.

Bias due to laboratory procedures

Measurement error

As mentioned above, measurement variation will occur whenever measurements are taken. Besides random variation, systematic errors in measurement may occur and lead to false-negative or false-positive results. Systematic errors generally result in either over- or underestimation of the overall proportion of resistance. Systematic measurement error occurs when laboratory procedures are improperly applied (e.g. plating a too large inoculum), when inadequate laboratory materials are used (e.g. poor quality growth media or expired antimicrobial disks), or when automatic systems are damaged or not properly calibrated.

Correct species identification may be important for the interpretation of resistance levels, as some species are more clinically relevant than others, and their capacity to acquire resistance or to be intrinsically resistant varies. Sometimes tell-tale signs indicate problems with species identification. For example, a high proportion (> 5%) of ampicillin resistance in *E. faecalis* suggests misclassification of *E. faecium* as *E. faecalis*.

A laboratory quality management system and regular application of internal quality assurance procedures allows the timely detection and correction of systematic errors in laboratory procedures. National auditing and accreditation schemes in conjunction with external quality assurance programmes ensure that laboratories conform to national quality standards.

Importantly, specific highly resistant microorganisms or exceptional antimicrobial resistant phenotypes (e.g. carbapenem-resistant Enterobacteriaceae) may need to be confirmed by additional testing, to assess whether they are true findings or may be due to laboratory error. This double checking of results is important because the finding of these types of organisms may have serious consequences for empiric antimicrobial therapy, and for infection prevention and control policies.

Laboratory standards

To ensure reliable results (Box 1), it is important that AST is done according to well developed and scientifically grounded standards. Both EUCAST and CLSI provide comprehensive methodological standards for routine AST, confirmatory testing and its interpretation. Because laboratory methods and interpretive criteria (i.e. clinical breakpoints) may differ between standards and will change over time, they may lead to incomparable results when assessing trends, and comparing results from laboratories or countries using different standards may be problematic.

Importantly, susceptibility to all indicated antimicrobials should be tested for each isolate included in surveillance. Differential or sequential test ordering, for example, only testing carbapenems if there is resistance to third-generation cephalosporins, will lead to overestimation of resistance proportions.

Box 1. Definitions

Active surveillance – surveillance based on active case-finding, testing and reporting, special efforts are made to identify all cases of disease

Bias – systematic deviation of results from the truth

Data generating process – procedures and routes by which data reach a database – all steps from identification of patients to be sampled, via laboratory procedures to storing and selection of results for analysis.

Passive surveillance – surveillance based on the collection of routinely available data or notification of disease cases by health workers, no special efforts are made to identify all cases of disease

Reliability – also known as reproducibility, the degree in which results of a measurement would be the same a next time the measurement was carried

Representativeness – also known as generalisability, the degree to which results of surveillance are true for the population of interest

Sampling bias – systematic error due to the methods or procedures used to sample or select the study subjects, specimens, or items –or– systematic differences between participants and non-participants

Target population – the group at which inference from the study is targeted; in the case of CAESAR, patients presenting with a bloodstream infection

Bias from data aggregation and analysis procedures

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 cultured more than once. Inclusion of repeat isolates from an individual patient when calculating the proportion resistance will result in overestimation, due to overrepresentation of resistant isolates. To prevent this in CAESAR, only the first isolate per microorganism per person per year is collected, as is the convention when doing surveillance.

When interpreting AST results in practice, for the purpose of reporting results to the clinic, expert rules are often used. For example, if *S. aureus* is resistant to ceftaxime it is reported as resistant to all beta-lactam antimicrobials. Different laboratories or national surveillance systems may use different expert rules, which may make comparison between laboratories/countries problematic. To prevent varying practice from biasing the results, i.e. to standardize the interpretation, results – susceptible, intermediate or resistant – are collected for all bacteria-antimicrobial combinations tested, and derived resistance is inferred during data analysis.

Recommended reading

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World Health Organization Regional Office for Europe

UN City, Marmorvej 51
DK-2100 Copenhagen Ø, Denmark
Tel.: +45 45 33 70 00 Fax: +45 45 33 70 01
Email: contact@euro.who.int
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