

# A New Instrument for Microbial Epidemiology

## Empowering Antimicrobial Resistance Data Analysis

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# Preamble

This is the integral PhD thesis ‘A New Instrument for Microbial Epidemiology’ (DOI 10.33612/diss.177417131) by Matthijs S. Berends, which was defended publicly at the University of Groningen, the Netherlands, on 25 August 2021.

All texts were copied from the printed version ‘as is’; no modifications were made.

## **Short summary (250 words)**

Treating infectious diseases requires insights into the microorganisms causing infectious diseases. Antimicrobial resistance (AMR) in microorganisms limits treatment possibilities and poses an enormous healthcare problem worldwide. The spread and AMR patterns of microorganisms, risk factors for infection, and preventive and control measures of infectious disease are studied within the field of Microbial Epidemiology, a cross-over field between Epidemiology and Clinical Microbiology. For analysing the spread and AMR patterns of microorganisms, however, no standardised method previously existed. This thesis showcases the development and applied use of a new instrument to analyse AMR data: the AMR package for R. From multiple viewpoints, the AMR package and its advantages are put into perspective: from a technical viewpoint, from an infection management viewpoint and from a clinical viewpoint. These combined provide a common ground for comprehending what the AMR package could yield in the field and how it can set a new empowered starting point for future applications of microbial epidemiology, in clinical and research settings alike. This thesis subsequently elaborates on these multiple viewpoints by illustrating the use of this new instrument in epidemiological research projects in the Dutch-German cross-border region to better understand the occurrence and AMR patterns of microorganisms on a (eu)regional level. In conclusion, this thesis shows the added value of a consistent data-analytical instrument to prepare and analyse AMR data in a full-region approach, that can also be used in clinical settings to obtain novel insights on AMR patterns.



# Colophon

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Berends MS, Luz CF, Sinha BNM, Glasner C<sup>‡</sup>, Friedrich AW<sup>‡</sup>  
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**Alphabetical list of published work**

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\* Equal contribution

‡ Equal contribution

# Chapter 1

## General Introduction

### 1.1 Microbial epidemiology

Epidemiology is the medical scientific field that investigates all the factors that determine the presence or absence of diseases and disorders. While many subspecialties within this field exist nowadays, such as veterinary epidemiology and cardiovascular epidemiology, its development started with an infectious disease. Between 1846 and 1860, the world endured the third cholera pandemic, taking assumably millions of lives <sup>[1]</sup>. The year 1854 was considered the worst year, when 23,000 people died in the United Kingdom, out of 16 million inhabitants (0.14%) <sup>[2]</sup>. As a side note, this is still quite less than the 146,000 UK deaths due to COVID-19 out of 56 million inhabitants (0.26%) until March 2021 <sup>[3]</sup>. But 1854 was also the year that the basis was laid for the field of epidemiology by John Snow, an English physician and hygiene specialist.

At the time of a local cholera outbreak at the Broad Street in London in that year, Snow did not know the exact source of cholera and called it ‘cholera poison’ in a book he published in 1856 <sup>[4]</sup>. Interestingly, the Italian Filippo Pacini managed to isolate the bacterium causing cholera, *Vibrio cholerae*, in 1854 – the same year that Snow investigated the outbreak <sup>[5]</sup>. Although it was not until 1884 that *V. cholerae* was formally given its name by the German Robert Koch <sup>[6]</sup>.

In his book about the ‘cholera poison’, Snow famously wrote <sup>[4]</sup>:

There is no doubt that the mortality was much diminished, as I said before, by the flight of the population, which commenced soon after the outbreak; but the attacks had so far diminished before the use of the water was stopped, that it is impossible to decide whether the well still contained the cholera poison in an active state, or whether, from some cause, the water had become free from it.

For this reason, Snow hypothesised that the local outbreak was caused by poisoned water coming from a water pump. To investigate the number of cases, he drew one of the most well-known data visualisations in epidemiology, Figure 1.1 (top). In this then-novel form of data visualisation, he counted the cases per household and denoted them as stacked rectangles. This resulted in his conclusion that there had been no particular outbreak or prevalence of cholera in that part of London except among the persons who were in the habit of drinking the water of one specific water pump: the one on Broad Street. The handle of the pump was removed on the day following his briefing to the local government, leading to an end of the outbreak.

With the advancements in information technology, heatmaps would nowadays be a more effective way to visualise geographic trends, Figure 1.1 (bottom). Using modern map data as illustrated, the incredible accuracy of Snow's drawing of London from 167 years ago is also highlighted. The type of investigating geographic trends in health and disease is nowadays known as *spatial epidemiology*.



Spatial epidemiology is one example of the many different specialities in the field of epidemiology. Another example is the direct consequence of Snow's work: infectious disease epidemiology, which has developed widely since the nineteenth century and has become the de facto standard for researching diseases and their health effects caused by pathogens (i.e., bacteria, viruses and fungi). Since this speciality concerns pathogens, it is a domain shared by the fields of epidemiology and clinical microbiology (Figure 1.2). Moreover, infectious

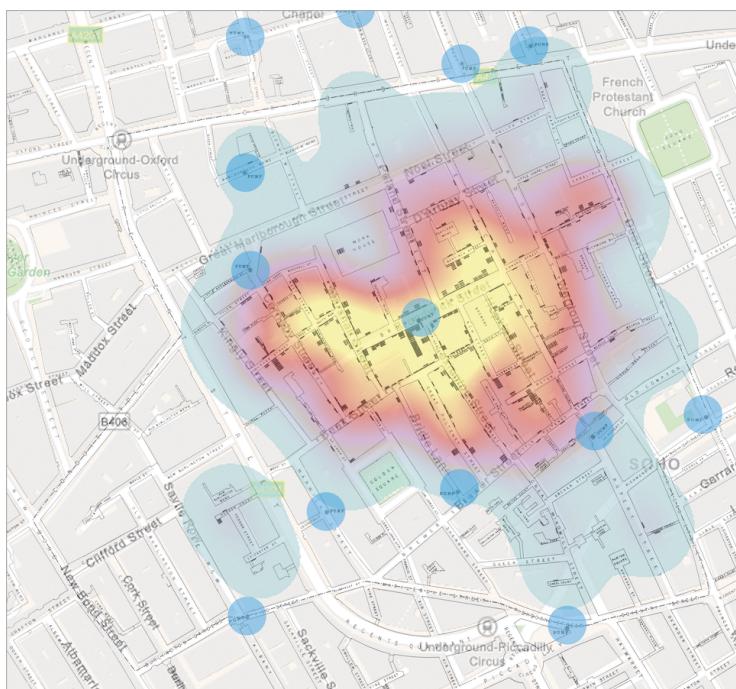


Figure 1.1: Visualisations of the ‘Broad Street cholera outbreak’ in London in 1854. Top: original map as drawn by John Snow. Bottom: Snow’s original map with a self-made heatmap visualisation overlay, based on the geographic position of the cases. The blue circles ( $n = 13$ ) indicate the location of the water pumps.

disease epidemiology can be split into two subspecialties: clinical (infectious disease) epidemiology and microbial epidemiology. The former focuses on the properties of the disease (such as the burden of disease caused by infection, or the disease-related mental and financial costs), while the latter focuses on the properties of the pathogen (such as the credibility of its source, antimicrobial resistance and pathogenicity).

Applying microbial epidemiology was barely possible in the days of John Snow, for the lack of scientific knowledge about pathogens and the lack of advancement in information technology. Antibiotics were not discovered yet, the cause of cholera was undetermined, and scientists had no clue about the infectivity and pathogenicity of different bacteria. However, what John Snow did in 1854 ‘clinical epidemiologically’, is in essence quite equal to what we currently do on a large scale during the COVID-19 pandemic. Information technology required to attain this large scale has brought us not only the possibilities to look beyond regional, national and international borders but to observe, analyse and understand pandemics in real-time. Methods we develop and use today can be implemented on the other side of the world tomorrow. This is an important advantage in modern infectious disease epidemiology, as is also illustrated in this thesis.

Microbial epidemiology has an important focus on observing and analysing (1) the microorganisms that cause infections and the human site of origin, (2) the intrinsic or acquired antimicrobial resistance they manifest, and (3) their infectivity and pathogenicity. As any type of microorganism – bacteria, viruses and fungi (including yeasts) – can cause infections in humans, microbial epidemiology is not limited to a certain type of microorganism. Nonetheless, there tends to be a stronger focus on bacteria and fungi, which are more easily isolated at a clinical microbiology laboratory than viruses and can be tested for phenotypical antimicrobial resistance in a routine diagnostic setting. Based on these diagnostic findings, treatment guidelines are developed and evaluated. This in itself urges microbial epidemiology to be employed in a routine setting as well, to make sure that treatment guideline development continually has a solid epidemiological basis.

## 1.2 Antimicrobial resistance in microorganisms

The antimicrobial resistance (AMR) that manifests in bacteria and fungi, is central within the diverse field of microbial epidemiology. It occurs when microorganisms develop mechanisms that protect them from the effects of antimicrobial agents, such as antibiotics [7]. AMR occurring specifically in bacteria is often termed antibiotic resistance (ABR). An important distinction should be made between intrinsic AMR (that is, AMR inherently present in certain microbial species as a distinctive property of that species) and acquired AMR (that is, AMR present in some strains of a certain microbial species induced by the presence of an antimicrobial agent). Infections caused by microorganisms

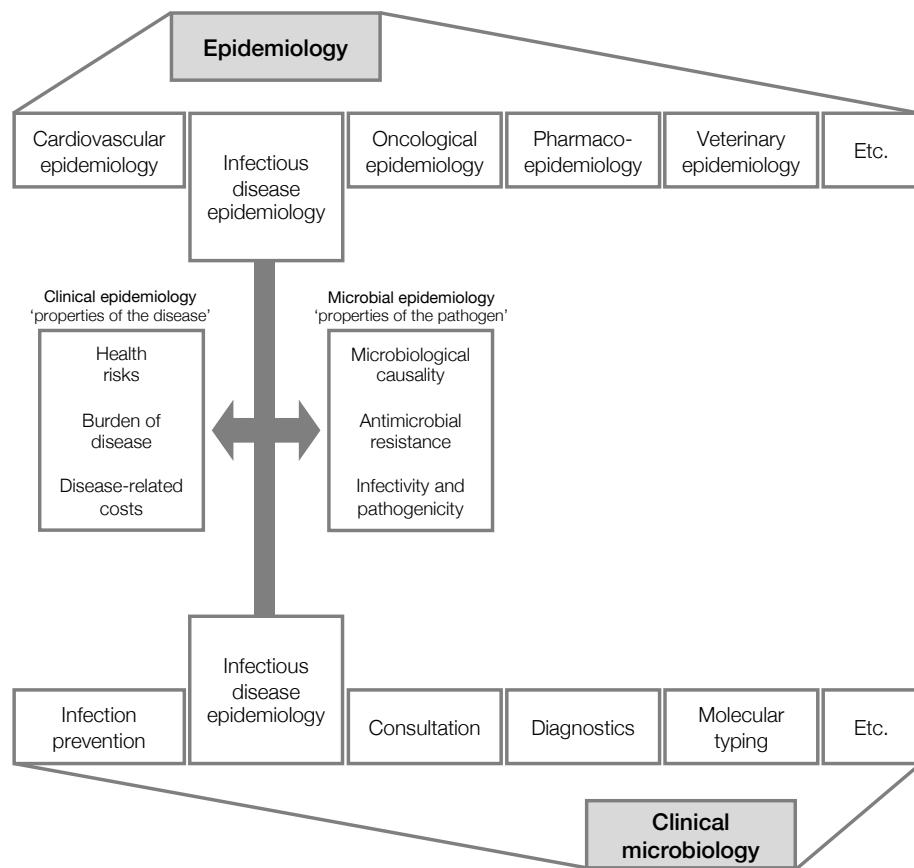


Figure 1.2: Overview of the diverse sections and subspecialties of epidemiology and clinical microbiology and their common field: infectious disease epidemiology. Microbial epidemiology can be considered to be a subspecialty of infectious disease epidemiology.

that are resistant to one or more antimicrobial agents cannot be treated with those antimicrobial agents anymore.

AMR is a global health problem and of great concern for human medicine, veterinary medicine, and the environment alike. It is associated with significant burdens to both patients and health care systems. Current estimates show the immense dimensions we are already facing, such as claiming at least 50,000 lives due to AMR each year across Europe and the US alone [8]. Although estimates for the burden through AMR and their predictions are disputed by some, the rising trend is undeniable, thus calling for worldwide efforts to tackle this problem [9,10]. For this reason, laboratory diagnostics are of utmost importance for generating AMR results that can be used to acquire new or improved AMR insights by conducting microbial epidemiology.

### 1.2.1 Laboratory diagnostics

From clinical illness alone (such as fever, redness, swelling, pain, and loss of function), it is impossible to determine whether the microorganism causing the infection is drug-resistant; it requires laboratory diagnostics to measure AMR. For decades, clinical microbiological laboratories have been using techniques where a defined amount of a microbial isolate is brought unto the medium of an agar plate [11]. This technique is called the ‘disk diffusion test’ and was first used by Dutch botanist Martinus Beijerinck in 1889 to study the effect of auxins (a class of plant hormones) on bacterial growth [11,12]. The technique has been further developed and refined by the American microbiologists William Kirby and Alfred Bauer in 1959 and 1966, leading to this test technique sometimes being referred to as the ‘Kirby-Bauer test’ or ‘KB test’ [13,14]. To perform the test, small filter paper disks containing a specified concentration of different antimicrobial agents are laid on the agar medium containing the microorganism, which is subsequently incubated for 18 to 24 hours at a specified temperature. During the incubation, the antimicrobial agent (antibiotic or antifungal) will radially diffuse over the agar, leading to high antimicrobial concentrations near the disk and low antimicrobial concentrations away from the disk. A disk typically has a diameter of 6 millimetres. After the incubation, the growth inhibition zone around the disk can be measured with a ruler. The wider the growth inhibition zone, the lower antimicrobial concentrations are required for the microorganism to inhibit growth. The narrower the growth inhibition zone, the higher antimicrobial concentrations are required for the microorganism to inhibit growth. The range of a disk diffusion test result is typically 6 to 50 millimetres.

Although disk diffusion tests are being widely used in many areas, some laboratories have replaced them with an automated incubator allowing colourimetric detection of CO<sub>2</sub> produced by growing microorganisms in the presence of antimicrobial agents [15–17]. Growth is subsequently optically measured for different concentrations and different antimicrobial agents. The concentration that inhibits at least 99.99% growth of the microorganism, is denoted the minimum inhibitory concentration (MIC) and is typically expressed in milligrams per litre

(mg/L). These incubators are referred to as antimicrobial susceptibility testing (AST) devices. AST devices allow for timely and reproducible results. Yet, the cartridges used for this type of instrument have a limited number of wells to test different manufacturer-set concentrations and types of antimicrobial agents. Since this limitation thus disallows testing for any desired concentration, MICs are often capped at a minimum or maximum value. For example, an actual MIC could be 128 mg/L, although the highest available concentration on a cartridge could be 32 mg/L. In such cases, the MIC will be reported as 32 mg/L. This is a technical limitation of colourimetric detection of CO<sub>2</sub> production as a test technique, which brings important disadvantages for microbial epidemiological analyses. Capped values (such as 0.0125 mg/L and 32 mg/L) hinder comparison with previous findings or findings from other laboratories as they might conceal the true MICs. Furthermore, different cartridges may be used for bacteria isolated from different specimen types (such as urine or blood), which can yield different ranges of the resulting MICs. For example, an isolate of *Staphylococcus aureus* from a urinary tract infection could be tested for many concentrations of only a few orally available antibiotics using cartridge A, while an isolate of *S. aureus* from a complex surgical wound could be tested for only a few concentrations of many intravenously available antibiotics using cartridge B. Consequently, the MIC of e.g., ciprofloxacin could be reported as 0.0625 mg/L using cartridge A, while it could be reported as 0.125 mg/L using cartridge B, even when the *S. aureus* isolates are identical. This makes it hard to compare results in epidemiological data analyses as the data availability can (unknowingly) be unequal, potentially affecting the outcome of any AMR data analysis.

### 1.2.2 Interpretation of raw results

When raw AMR testing results are available, they are not yet suitable for reporting back to clinicians. The growth inhibition zones of disk diffusion tests and the MICs from the colourimetric detection tests need interpretation to consider an antimicrobial agent suitable for treatment. Typically, AMR is interpreted and reported as either (a tri-form abbreviated as 'RSI'):

- R = resistant. A microorganism is categorised as 'resistant' when there is a high likelihood of therapeutic failure even when there is increased exposure.  
Exposure is a function of how the mode of administration, dose, dosing interval, infusion time, as well as distribution and excretion of the antimicrobial agent will influence the infecting organism at the site of infection.
- S = susceptible. A microorganism is categorised as 'susceptible' when there is a high likelihood of therapeutic success using a standard dosing regimen of the agent.
- I  
(according to CLSI) I = intermediate. A microorganism is categorised

as ‘intermediate’ when there is an unsure likelihood of therapeutic success. Additionally, CLSI considers a susceptible dose-dependent (SDD) category for certain drug and organism combinations, for which the susceptibility of an isolate depends on the dosing regimen used.

(according to EUCAST) I = Susceptible, increased exposure. A microorganism is categorised as such when there is a high likelihood of therapeutic success because exposure to the agent is increased by adjusting the dosing regimen or by its concentration at the site of infection.

For this interpretation of raw AMR test results, international guidelines exist. The most often applied guidelines are supplied by the Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) [18,19]. In Europe, an increasing number of clinical laboratories apply EUCAST guidelines, as it was shown that the coverage of EUCAST guidelines among these laboratories was 73.2% in 2013, and only a few European countries did not use the EUCAST methodology in 2019 [20,21]. According to the World Health Organisation (WHO), guidelines from CLSI and EUCAST are adopted by 94% of all countries reporting AMR to the Global Antimicrobial Resistance Surveillance System (GLASS) of the WHO [22].

Generally, AMR is defined as the proportion of resistant microorganisms (R) among all tested microorganisms of the same species (R + S + I). The CLSI and EUCAST guidelines define the interpretations for the most common combinations of pathogenic microorganisms and antimicrobial agents. For example, the EUCAST 2021 guideline considers ciprofloxacin against *Escherichia coli* to be susceptible when either the MIC is at most 0.25 mg/L or when a diffusion disk with 5 µg has a growth inhibition zone of at least 25 millimetres (Figure 1.3).

In 2017, EUCAST implemented the area of technical uncertainty (ATU) for certain microbial species/antibiotic combinations, to warn laboratory staff that the interpretation of routine susceptibility testing is uncertain [23]. For example, disk diffusion results from the combination of any species in the order of Enterobacterales with amoxicillin/clavulanic acid are considered unreliable for a zone diameter of 19–20 mm in the latest EUCAST interpretation guideline [24]. EUCAST advises to rerun the test, perform an additional test, or to report this uncertainty with a clear warning [23].

To mitigate the risks of laboratories reporting erroneous susceptibility results, CLSI and EUCAST guidelines are also provided as “expert rules” in the previously mentioned AST devices, which helps to ensure compliance with guidelines and standards, increasing the quality of AMR data [25].

Analysing AMR data, such as raw MICs and antimicrobial interpretations (‘RSI’), is tedious and complex, especially when evaluating cumulative AMR reports [26]. Nonetheless, it is essential to monitor up-and-coming AMR trends at the local and regional level to support clinical decision-making, infection control interventions, and AMR containment strategies [27,28]. AMR data analysis

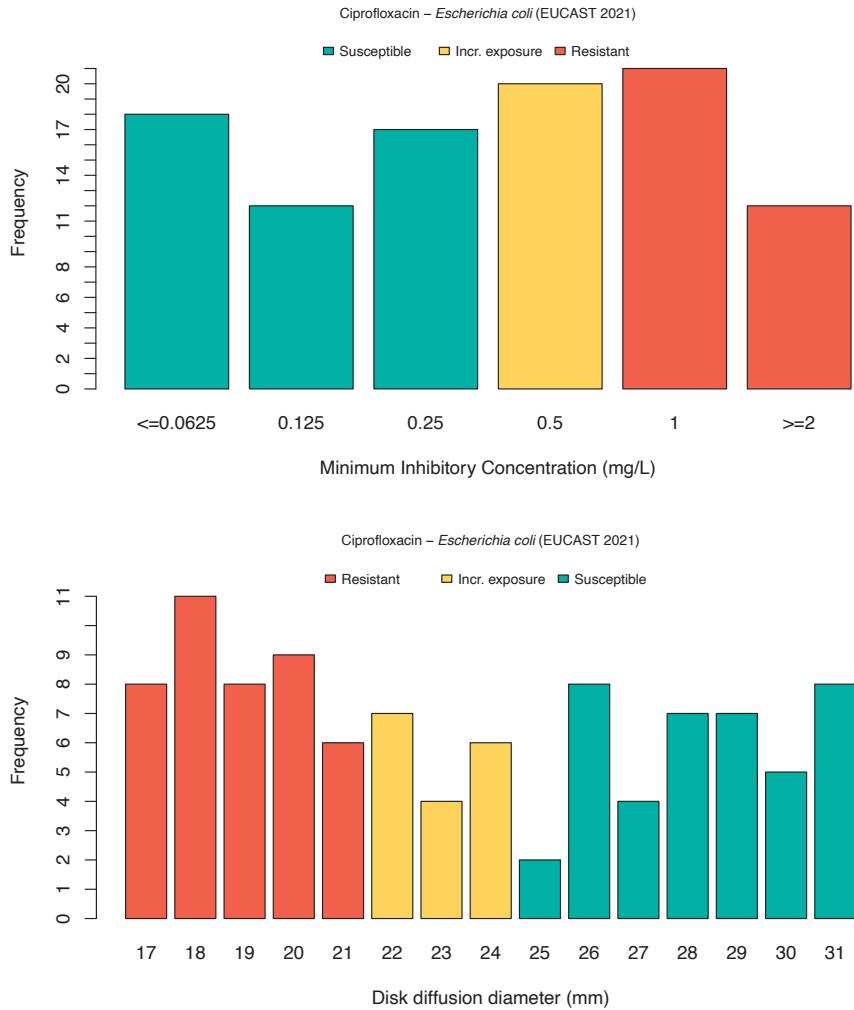


Figure 1.3: Interpretation of 100 random minimum inhibitory concentrations (top) and 100 random disk diffusion growth inhibition zones (bottom) of ciprofloxacin in \**Escherichia coli*\*, interpreted using colours according to the EUCAST 2021 guideline. These plots were generated with the AMR package for R.

has been challenged by poor comparability of antimicrobial susceptibility statistics between institutions because of the diversity of calculation methods [26]. Moreover, many laboratories have used simplistic calculation approaches, with a strong tendency to overestimate drug resistance rates [26]. In the first ten years of this century, it was shown that this was primarily attributed to the lack of correction for duplicate isolates [29–31].

In an attempt to overcome this, CLSI started in 2002 with developing guidelines to recommend epidemiologically sound workflows for the analysis and presentation of AMR results and trends, with their fourth and currently latest version released in 2014 [32]. These guidelines comprise advice on the inclusion of a minimum number of isolates, the choice of antimicrobial agents to analyse, and the presenting of numbers and percentages of AMR. In 2007, Hindler *et al.* evaluated the then-latest version of this guideline [26]. They concluded that although CLSI provided a comprehensive collection of suggestions, only a few publications had implemented these practical recommendations. Nevertheless, it continuously provides a theoretical basis for microbial epidemiological analyses but lacks suggestions of how these theoretical recommendations can be implemented practically or what kind of software would be suitable to analyse AMR data and, more specific, AMR data about multi-drug resistant organisms.

### 1.2.3 Multi-drug resistant organisms

Multi-drug resistant organisms (MDROs) are microorganisms that acquired AMR to at least one antimicrobial agent in multiple antimicrobial categories. Because of MDROs, there are countries in many parts of the world where antimicrobial treatment is ineffective in more than half of all patients [33]. Common MDROs include vancomycin-resistant enterococci (VRE), methicillin-resistant *Staphylococcus aureus* (MRSA), extended-spectrum -lactamase (ESBL) producing Gram-negative bacteria such as *E. coli* and *Klebsiella pneumoniae*, carbapenemase-producing Gram-negative bacteria, third-generation cephalosporin (3GC) resistant Gram-negative bacteria and carbapenemase-producing Gram-negative bacteria.

In 2012, MDROs were formally categorised into different degrees of severity in favour of international comparison purposes [34]. Multi-drug resistance (MDR) was defined as acquired AMR to three or more antimicrobial categories, extensive drug resistance (XDR) was defined as acquired AMR to all antimicrobial agents except in two or fewer antimicrobial categories, and pan-drug resistance (PDR) was defined as acquired AMR to all antimicrobial agents in all antimicrobial categories [34]. MDR among microorganisms is very common, PDR is very uncommon [7,33,35]. In 2014, the WHO published a report in which they performed five systematic reviews involving 221 studies with a special focus on MDR bacteria (defined as MRSA, 3GC/fluoroquinolone-resistant *E. coli*, and 3GC/carbapenem-resistant *K. pneumoniae*) [36]. The outcomes of this report underlined the increasing necessity of surveillance programs.

### 1.2.4 Surveillance programs

With the current WHO surveillance program GLASS, the overall coverage of AMR is continuously being monitored for most countries of the world [37]. For Europe, the prevalence of AMR on the country level is monitored by national surveillance programs that share their data with the European Centre for Disease Prevention and Control (ECDC), an agency of the European Union [38]. Their surveillance program European Antimicrobial Resistance Surveillance Network (EARS-Net) is the largest publicly funded system for AMR surveillance in Europe. Public access to descriptive data (maps, graphs and tables) are available through the ECDC Surveillance Atlas of Infectious Diseases [38], which was also consulted for multiple studies in this thesis. While the ECDC estimated in 2009 that bacterial infections caused by MDROs were responsible for 25,000 extra deaths per year [39], others found that there is a large discrepancy between the real count of deaths attributable to MDROs and the subsequent alarmist predictions, based on data from over 500 studies [35].

Although surveillance programs allow for signalling significant differences and shifts in AMR rates, additional AMR data analyses and AMR surveillance studies are strict requirements to fully understand the continuous development in AMR rates as there is no “ideal” surveillance system covering all aspects [28]. Nonetheless, the desire to continuously monitor, analyse, model and predict AMR, has led to the increased development and use of local, regional, national and international surveillance systems [27]. Critchley *et al.* have inventoried the requirement set by different types of users (Table 1).

On the local level, clinical microbiology laboratories should communicate AMR surveillance data to healthcare providers in an understandable manner. Since MDROs can migrate between healthcare institutions, countries and continents by migrating people, local healthcare providers should be aware of local, regional, national and international surveillance program implementations and their ensuing results on AMR. On the other hand, such surveillance program implementations should be well-designed, well-maintained, longitudinal, and involve an appropriate collaboration with local laboratories over time [27].

Table 1. Uses of antibiotic resistance surveillance system data by hospitals, university researchers, pharmaceutical companies and governments. From Critchley *et al.*, 2004 [27].

As an example, ISIS-AR (Infectious disease Surveillance Information System for Antibiotic Resistance) is a Dutch national surveillance program, for which a large number of the Dutch clinical microbiology laboratories provide anonymised data on AMR to the National Institute for Public Health and the Environment (Rijksinstituut voor Volksgezondheid en Milieu, RIVM) [40]. In Germany, ARS (Antibiotic Resistance Surveillance) is a similar laboratory-based national surveillance program, that attempts to enable differential statements according to structural characteristics of health care and regions [41,42]. Both these national surveillance programs provide data for EARS-Net and GLASS of the

Uses of antibiotic resistance surveillance system data	Users of antibiotic resistance surveillance data				
	Hospitals	Community healthcare providers	University researchers	Pharmaceutical companies	Governments
Guide patient therapy	X	X	-	-	-
Identify trends in antibiotic resistance; assess the magnitude of new resistance threats; follow the dynamics of resistance trends	X	X	X	X	X
Detect new resistance mechanisms	-	-	X	X	-
Monitor impact of empirical prescribing	X	X	-	-	X
Monitor effects of infection control interventions	X	-	-	-	-
Identify outbreak of antibiotic-resistant organisms	X	-	-	-	-
Detect bioterrorist events	X	X	-	-	X
Monitor antibiotic resistance during the product development cycle	-	-	X	X	-
Identify needs for new antibiotics: monitor the needs for targeted-spectrum antibiotics	-	-	X	X	-
Identify the need for new diagnostic tests and unmet medical needs	-	-	X	X	-
Education and continuing education on antibiotic resistance	X	X	X	X	X
Strategic information to support new antibiotic drug target development	-	-	X	X	-
Identify high-profile isolates for antibiotic screens to guide structure-activity-relationship strategies for novel targets	-	-	X	X	-
Antibiotic resistance modelling	-	-	X	X	
Benchmark the activity of new antibiotics; pre- and post-regulatory approval	-	-	-	X	X
Regulatory agency submissions such as new drug applications (NDAs) or other regulatory documents	-	-	-	X	X
MIC interpretative criteria submissions (breakpoint determinations) to government or regulatory agencies	-	-	-	X	X

WHO [37,43].

### 1.3 Data analysis using R

In academia, the free and open-source statistical language R is an increasingly popular tool for analysing study results and developing new scientific methods, especially in medical fields such as human genetics, health decision sciences, and proteomics [44–47]. Even more so, a new type of study seems to currently arise where researchers from different medical fields publish tutorials on how to acquire new insights using R as a programming language [48–50]. In 2020, R ranked 8th in the TIOBE index, a global initiative to measure the popularity of programming languages, while it ranked 73rd in 2008 [51].

R was developed for statistical computing and graphics supported by the R Foundation for Statistical Computing [52,53]. It is freely available under the GNU General Public License v2, meaning that it may be used for both private and commercial purposes in any way, but not for patent purposes. As a statistical package, it is comparable to the proprietary software programs Stata, SAS and SPSS [54]. However, as opposed to these proprietary software programs, R has an open file format and can read data from any source, including files from other software programs, and websites. Moreover, the ‘base’ functions of R are extendible by users who develop so-called packages for R. The Comprehensive R Archive Network (CRAN) that hosts and maintains R through the R Foundation for Statistical Computing, accepts package submissions from users and subjects users to a peer-review submission process and a strict repository policy [53,55]. As of May 2021, the CRAN package repository features 17,671 available packages.

Not only the popularity of using R has increased over the last decade. The number of developed packages has also increased strongly over the last years, especially since 2016 (Figure 1.4). This is probably attributed to a rather new integrated desktop environment (IDE) to use R, called RStudio [56]. RStudio is also the name of the corporation that developed the RStudio IDE and authored the so-called tidyverse, a collection of R packages (such as dplyr and ggplot2) that are specifically designed to ease data importing, tidying, manipulating, visualising, and programming, as well as to improve code reading [57–59]. The tidyverse can be used for most data analytical tasks and has been the method of choice for numerous (clinical) studies, including those presented in this thesis.

For microbial epidemiology, no particular R packages were available to analyse phenotypic AMR test results as of 2017. One R package that provides approaches to work with disk diffusion zone diameters and MICs from environment samples started development in 2018, but still has no released version as of May 2021 [60]. For ‘non-microbial’ infectious disease epidemiology, however, outbreaks and epidemics could already be analysed with dedicated packages in R [61–65]. Most of these packages were developed within RECON, the R Epidemics Consortium, that gathers experts in data science, modelling methodology, pub-

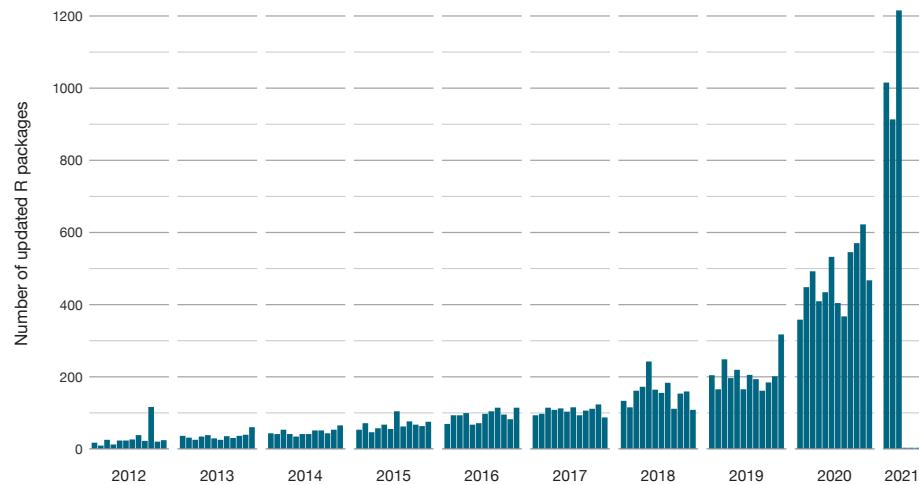


Figure 1.4: The number of R packages by date of the last update over the last ten years. Every bar represents one month. Every R package occurs once in this figure.

lic health, and software development to create the next generation of analytics tools in R for informing the response to disease outbreaks, health emergencies and humanitarian crises. Their R package EpiEstim is being used worldwide for calculating and presenting reproduction rates of SARS-CoV-2 during the ongoing COVID-19 pandemic, also by the Dutch National Institute for Public Health and the Environment (RIVM) [65,66].

## 1.4 Setting for this thesis

Studies within this thesis were geographically organised or initiated in the Northern cross-border region of the Netherlands and Germany, Figure 1.5. According to the German philosopher Liessmann, there are only national borders defined by humans, but no natural borders [67]. He explained that borders as man-made conventions are never absolute, but that it is always possible to cross them. Despite the existing territorial border, there are many similarities in the Netherlands and Germany today, but just as many and clear differences, especially concerning the healthcare sector. A German patient can become a patient in the Netherlands just as quickly as a Dutch patient can in Germany. Since pathogens know no borders, patient protection and infection prevention must not stop at borders [68]. The Netherlands and Germany have, among many other matters, apparent differences within the healthcare system in general and in terms of AMR, especially concerning MDRO definitions and infection prevention guidelines. To study these differences, INTERREG programs enable cross-border, transnational and interregional cooperation. INTERREG is one

of the central instruments in European cohesion and regional policy, with which the development differences between the European countries in the border regions should be reduced and economic cohesion strengthened. It aims to ensure that national borders are not an obstacle to the balanced development and integration of the European territory [69]. One of its programs, EurHealth-1Health, was a large research project that aimed to facilitate working together in battling AMR and MDROs and to empower sustainable collaborations across the border.



Figure 1.5: Geographic overview of three Euregio's that make up most of the Dutch-German cross-border region.

In the Northern Netherlands, five clinical microbiological laboratories together conduct the microbiological diagnostics for more than two million Dutch inhabitants in primary care, secondary care (non-university hospitals) and tertiary care (university hospital). Three of these five are regional non-profit laboratories: Izore in Leeuwarden (Friesland), Certe in Groningen (Groningen) and LabMicTA in Hengelo (Overijssel). The other two laboratories are hospital departments of the Isala hospital in Zwolle (Overijssel) and the University Medical Center Groningen. On the other side of the border in Germany, laboratories are more numerous, more centralised, often privatised, and organised on a different scale than in the Netherlands. This is largely due to a higher number of small hospitals in Germany compared to the Netherlands, which is inherent to the different healthcare structures. In 2018, Germany had 2.33 hospitals per 100,000 inhabitants (1 hospital per 43,010 inhabitants), while in the Netherlands this

was 0.68 hospitals per 100,000 inhabitants (1 hospital per 148,113 inhabitants), almost 3.5 times less [70–73].

These differences posed important reasons to research the effects of having different national guidelines regarding AMR (and MDRO interpretations) and screening guidelines, as is investigated in this thesis.

## 1.5 Aim of this thesis and introduction to its chapters

This thesis aims to present the development of a new instrument for microbial epidemiology – a new and open method for standardised AMR data analysis – while also providing applied examples of how this new instrument has empowered AMR data analysis in regional and euregional studies.

This thesis is presented in four sections.

**SECTION I** opens with a broad introduction to the usefulness and necessity of having timely diagnostic information in **chapter 2**. Diagnostic stewardship programs (DSP) are a requirement to gain answers instead of results, including those from a clinical microbiology laboratory. DSP is a multidisciplinary approach to gain the most benefit for the patient by democratising different medical specialities. In **chapter 3**, the usefulness and necessity of having a dedicated tool for microbial epidemiology are introduced, through the AMR package for R as a new instrument. It is explained why microbial epidemiology and its effects are hindering efforts to dispose of AMR trends and how the AMR package for R can compensate for this. This chapter was primarily intended for non-data-technical professionals who work in the field of infectious diseases, such as clinical microbiologists and infectiologists.

**SECTION II** outlines the working and implementation of the AMR package for R. It starts with explaining this newly developed instrument in **chapter 4**. In this methodological and technical paper, the working mechanisms of the AMR package for R are thoroughly described. It is demonstrated that the AMR package enables standardised and reproducible AMR data analyses, including the application of evidence-based rules, determination of first isolates, translation of various codes for microorganisms and antimicrobial agents, determination of (multi-drug) resistant microorganisms, and calculation of antimicrobial resistance, prevalence and future trends. This chapter was primarily intended for data-technical professionals who work in the field of microbiology, such as (infectious disease) epidemiologists and biostatisticians. For **chapter 5**, the AMR package was implemented in a newly developed web application to present the design, development, and testing of RadaR (Rapid analysis of diagnostic and antimicrobial patterns in R), a software app for infection management, and to ascertain whether RadaR can facilitate user-friendly, intuitive, and interactive analyses of large datasets in the absence of prior in-depth software or program-

ming knowledge. Subsequently, in **chapter 6**, we aimed at demonstrating and studying the usability of our developed approach and its impact on clinicians' workflows in a typical scenario. By comparing traditional software methods such as Excel and SPSS with an online implementation of our new instrument, we tried to establish the benefit of using dedicated tools in a clinical situation.

**SECTION III** provides real-life examples of how the new instrument was used in studies that focus on AMR data analysis, in the Northern Dutch region as well as the Northern cross-border region of the Netherlands and Germany. **Chapter 7** brings a thorough analysis of the occurrence and antibiotic resistance of coagulase-negative staphylococci (CoNS) in the Northern three provinces of the Netherlands, by analysing almost 20,000 antibiograms. Since 2013, all regional clinical microbiological laboratories make use of matrix-assisted laser desorption/ionisation time-of-flight (MALDI-TOF) mass spectrometry to identify microbial isolates to the species level. Using the AMR package for R, all relevant antibiotic results could be analysed for all different CoNS species that were found during the study period (2013-2019). In **chapter 8**, country-specific guidelines for determining MDROs in the Netherlands and Germany were compared in this border region. This was done by interpreting all isolates found on both sides of the border with the national guidelines from both countries. Major differences were observed, which also imply a strong challenge for healthcare personnel working in the border region. Isolate selection and MDRO determination on the Dutch side of the border was carried out using the AMR package. **Chapter 9** outlines the euregional epidemiology of methicillin-resistant *Staphylococcus aureus* (MRSA) by analysing results from 42 hospitals. MRSA colonisation, infection and bacteraemia rate trends were described from the Dutch-German border region hospitals between 2012 and 2016. Although measures for MRSA cases were similar in both countries, defining patients at risk for MRSA differed. For **chapter 10**, twenty-three hospitals in the Dutch-German border region participated in a prospective screening study for the determination of the carriage of multi-drug resistance on admission to intensive care units (ICU), including more than 3,000 patients. The screening compliance, hospital and ICU sizes, and outcome of AMR data analysis were compared between both sides of the border.

**SECTION IV** summarises the presented work and provides future perspectives.

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## **Chapter 2**

# **Diagnostic Stewardship: Sense or Nonsense?!**

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### **Abstract**

The right test at the right time for the right patient to answer the right questions and start the right treatment - many important decisions have to be made involving multiple medical specialists. The importance of appropriate and timely diagnostics guide this process (stewardship) can be obvious but is still often neglected in classic stewardship concepts of infection management. We describe the approach of a multidisciplinary, intertwined stewardship concept with a focus on diagnostics, where medical specialists in general and microbiologists in particular closely interact for optimal quality of care and patient safety in successful infection management. Diagnostics in medical microbiology laboratories are advancing fast with regards to new technologies and improved workflows. Yet, diagnostics in infection management is broader than this and covers many

clinical areas where communication and interaction are the key to make the best use of knowledge and expertise that all specialisms can contribute to patient care. These aspects are demonstrated in two cases of patients with prosthetic joint infections with two very different outcomes.

## 2.1 Introduction

Diagnostic stewardship or diagnostic stewardship programme (DSP), a trending topic in the field of medical microbiology and beyond. But what is this concept about, is it really so new and how is it incorporated into infection management? The term diagnostic stewardship was used in an opinion piece by Dik *et al.* which described various facets of infection management, the so-called integrated stewardship [1]. We want to highlight the diagnostic side of this model and describe its concept; diagnostics as a multidisciplinary bigger picture from admission to discharge.

Although the term DSP was first mentioned in an indexed PubMed article in 2016, articles on antimicrobial stewardship (ASP) have been appearing for 15 years (Figure 2.1).

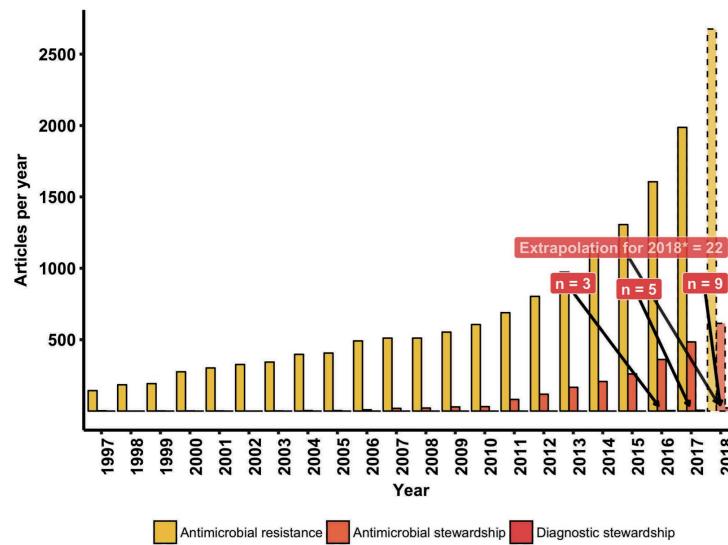


Figure 2.1: The increase of articles indexed in PubMed. Search strategies: 'antimicrobial stewardship'[Title/Abstract]; 'diagnostic stewardship'[Title/Abstract]; 'antimicrobial resistance'[Title/Abstract]. Source: <https://www.ncbi.nlm.nih.gov/pubmed/> (assessed: 2018-05-31). \* Extrapolation based on count from 2018-01-01 to 2018-05-31.

Nevertheless, the concept of DSP is neither intended to replace other stewardship concepts (in particular ASP) nor to be an alternative. DSP concerns

decision making and goes beyond microbiological diagnostics alone. Kahneman *et al.* [2] said about decision making:

We think, each of us, that we're much more rational than we are. And we think that we make our decisions because we have good reasons to make them. Even when it's the other way around. We believe in the reasons, because we've already made the decision. [2]

Adequate diagnostics should help us to prevent this kind of situation in medicine by providing a basis to make well-informed decisions. Defining a proper diagnosis is a complex process with several aspects. We believe that DSP is a concept that requires collaboration between different medical specialties for optimal infection management and quality of care. This can include reduced morbidity and/or mortality, unnecessary interventions or treatments, complications, and length of stay. We want to point out why and how DSP affects the entire diagnostic process and that it involves more than just results or turnaround times of microbiological tests. By comparing different patient cases, we want to demonstrate how DSP serves the most important purpose: improved patient care. This involves process optimisation as a basis as well as medical questions and decisions on the individual patient level.

This entire diagnostic process requires multiple decisions along the way of patient care. Guidance and communication on this path are essential because:

Intuitive diagnosis is reliable when people have a lot of relevant feedback. But people are very often willing to make intuitive diagnoses even when they're very likely to be wrong. [3]

Modern medicine is centred around evidence-based actions and tries to minimise the chance of mistakes while trying to keep the balance between the quality of care and the outcome on one hand and preventing collateral damage and costs on the other hand. In infection management stewardship activities can provide support and guidance in diagnosis and therapy. Physicians can be supported at the bedside to choose the right diagnostic test at the right time for the right patient. The same applies to therapeutic choices: the right treatment at the right time for the right patient in order to achieve the most optimal result. Naturally, these approaches to diagnostic and therapeutic support go hand in hand.

We outline two different case studies - fictitious but nevertheless realistic - of a patient with a prosthetic joint infection (PJI) in different scenarios and different outcomes. These examples underline how interdisciplinary stewardship can lead to a successful outcome for the patient and the physician.

### 2.1.1 Case 1

A 70-year-old woman was seen by the orthopaedic surgeon because of chronic pain in her hip prosthesis placed 3 years earlier. An X-ray showed signs of loosening of the prosthesis - an indication for revision surgery. C-reactive protein

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(CRP) was low (6 mg/L). The diagnosis of aseptic loosening was made, and the patient underwent revision surgery. To rule out low-grade infection, antibiotic prophylaxis was administered only after intraoperative tissue biopsies had been taken for culturing and histology. Cutibacterium acnes (formerly Propionibacterium acnes) was isolated from one out of five tissue biopsies (semi-quantitative <1+). Histology showed no indication of inflammation. The positive culture was considered contamination by the attending clinical microbiologist and the patient was discharged without further antibiotic therapy. However, during outpatient follow-up, the patient complained about persistent stiffness of her hip. Three years later, the patient presented again with recurrent loosening of the prosthesis and the presence of a fistula around the surgical site. A second revision intervention was necessary. Due to poor bone quality and poor soft tissue, multiple revisions were needed. Multiple intraoperative tissue biopsies revealed Cutibacterium acnes with the same antibiogram as three years earlier together with a methicillin-sensitive *Staphylococcus hominis*. The patient was given a cement spacer which made her temporarily immobile and was treated with a high dose of flucloxacillin intravenously. She was discharged with clindamycin per os and re-admitted several months later for reimplantation of the definitive prosthesis. After eight months of revalidation the functional result was poor. The patient permanently walks with support of a cane.

Figure 2.2 shows the course of the disease of this patient in which the decision moments are shown in circles. The potential stewardship zone shows the moments when a different action could/should have been taken.

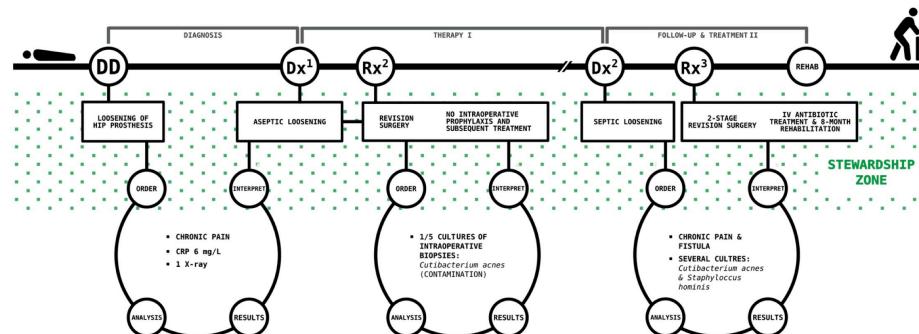


Figure 2.2: The first case.

The outcome for this patient was certainly not optimal. To illustrate how infection management with stewardship elements can improve the quality of care, a second case of the same patient with a PJI follows. Several additional diagnostic steps were performed (shown in bold) underlining the need for collaboration in stewardship activities including antimicrobial stewardship, of course, and how this affects clinical outcome and hospitalisation.

### 2.1.2 Case 2

A 70-year-old woman was seen by the orthopaedic surgeon because of chronic pain in her hip prosthesis placed 3 years earlier. An X-ray showed signs of loosening of the prosthesis - an indication for revision surgery. C-reactive protein (CRP) was low (6 mg/L). The radiologist was consulted to reassess the X-ray taken a year earlier. This image already showed subtle signs of radiolucency around the head and neck of the prosthesis making a mechanical cause of detachment less likely. Synovial fluid was punctured to rule out septic loosening of the prosthesis. The synovial fluid culture remained negative and the leukocyte count was only slightly increased, but several biomarkers were positive suggesting infection (450 mg/L calprotectin and positive alpha-defensin). Subsequently, prior to revision surgery, several tissue biopsies were taken by the orthopaedic surgeon in a sterile environment. Cutibacterium acnes (formerly Propionibacterium acnes) was isolated from one out of five tissue biopsies (5-10 CFU/ml). Histology showed no indication of inflammation. During revision surgery, antibiotic prophylaxis was given prior to surgical incision and several tissue samples were taken for culturing (including sonication) of the prosthesis. Empirical treatment was initiated with high doses of amoxicillin. Due to the previous positive culture with Cutibacterium acnes, all intraoperative cultures were incubated for 14 days on the advice of the clinical microbiologist. C. acnes was found again in two of five tissue biopsies and also in the sonication fluid. These isolates showed the same antibiogram as the isolates from before revision surgery. The patient was then discharged and treated at home with 10 weeks of amoxicillin per os. She fully recovered within a few weeks.

Figure 2.3 shows the additional decisions compared to Figure 2.2. These lead to a better outcome for the patient through the implementation of stewardships. The differences with Figure 2.2 are shown in red.

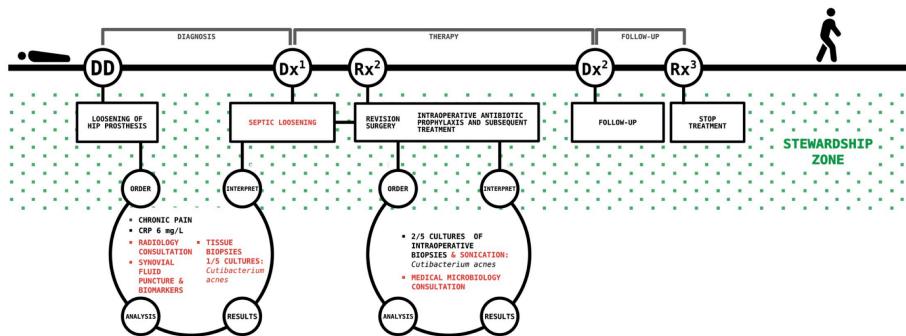


Figure 2.3: The second case.

## 2.2 The general concept

### 2.2.1 ‘Diagnostics’

The term diagnostics seems simple, but its various aspects are very diverse, as the cases above demonstrate. The second case emphasises the importance of stewardships and centres around facilitating an optimal care process through communication, crossing the boundaries of specialisms, and increasing awareness of the integral nature of successful infection management and optimal quality of care. Different physicians (involved in infection management) and their perceptions are reflected in this view on diagnostics. While some think of the entire process of diagnosing a disease, others think purely of the technical aspect in the lab as diagnostics (of their own speciality). This diversity underlines the importance of communication and collaboration across the boundaries of different medical specialties. The concept of stewardship is widely used to facilitate communication (and clinical decision making). Multiple attempts have been made to establish a clear definition of stewardship, but this has proved challenging [3,4]. Overall, most of these attempts have been made in the light of antimicrobial stewardship programmes (ASP) and are accompanied by terms such as responsibility, balance, due diligence, and management [3,4].

### 2.2.2 DSP in the microbiological laboratory

A medical laboratory usually only has added value if, in addition to the reporting and advice, the range of tests and the test technique meet the requirements of the applicant. The technical aspect of the medical microbiology laboratories has seen tremendous technological advances in recent years. Advanced developments such as sequencing as part the routine to identify isolate properties (e.g., resistance genes) and Matrix-Assisted Laser Desorption/Ionization Time of Flight (MALDI-TOF) mass spectrometry methods have recently revolutionized the laboratories [5-7]. In addition, many new and fast diagnostic assays such as point-of-care test (POCT) and molecular rapid diagnostic test (mRDT) have entered the market [8]. The progress is undeniable although integration into workflow, quality control, data storage and availability, added value, and clinical impact often still need to be evaluated.

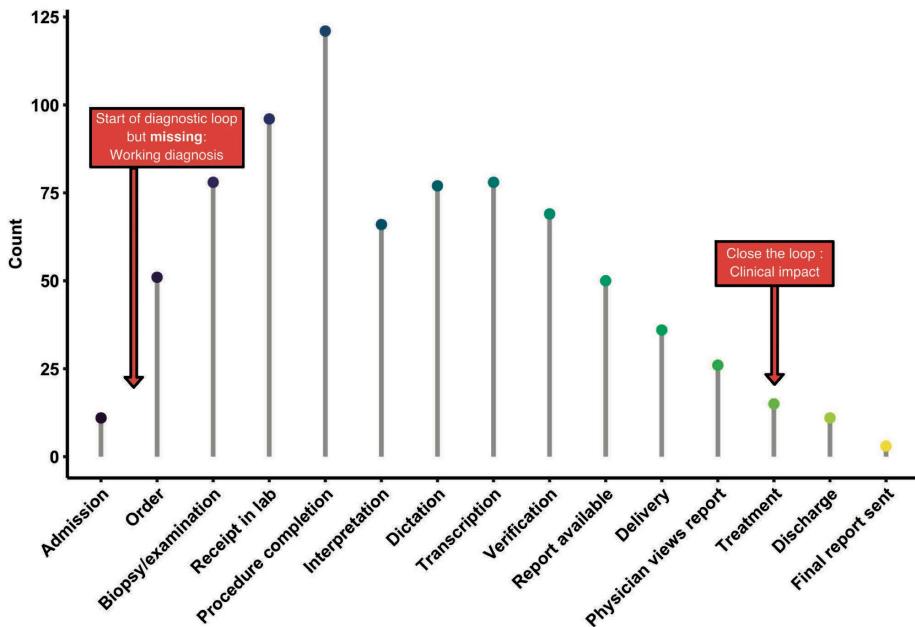
We embrace these developments but there are two aspects that are really essential for optimal quality of care. Both these aspects can be achieved through stewardship. Firstly, stewardship provides guidance for the appropriate choice of a customised diagnostic strategy for individual patients and patient groups in a specific setting. Guidelines and protocols for diagnostic and appropriate therapeutic choices are key elements in the development of this guidance or steering. A stewardship framework can form the basis for personalised decisions in individual patient care. It has already been demonstrated that new tests such as the aforementioned mRDT are most cost-effective for the diagnosis of bacteraemia when combined with an antimicrobial stewardship programme [9]. In addition, mRDT is associated with a significant reduction in mortality risk for

septic patients but only when combined with ASP [10].

Secondly, it is important to consider the entire information loop in a process-oriented way and not just focus on the time-to-result. Stewardship covers this loop and starts making choices at the bedside. In addition, the interpretation of test results and timely feedback are equally important in order to be able to make good, evidence-based, and rapid therapy adjustments when needed. For example, physicians considering starting non-prophylactic intravenous antimicrobial treatment should (almost) always take blood cultures before starting. Although this is standard care and described in international guidelines [11], compliance is only 30 to 50% [12, 13, Luz *et al.*; unpublished data]. Only through complete ‘loops’, from bedside to bedside, can better technology and improved work processes in microbiology laboratories be extended and made to work to their full potential.

### 2.2.3 DSP as process optimisation

Turnaround times (TAT) are a commonly used but poorly defined term in many areas. In a systematic review, a total of 61 different TAT definitions (out of a total of 151) were found to be used in several clinical areas [14]. Of those, only 10 definitions cover the time from test order placement to the time at which the results are being viewed by the ordering physician (Figure 2.4).



Adapted from: Breil B, Fritz F, Thiemann V, Dugas M. BMC Med Inform Decis Mak. 2011.

Figure 2.4: Time points mentioned in TAT definitions.

Nevertheless, even the order of a test is a decision within a diagnostic loop and should be taken into account when time is measured. We are convinced that infection management can help to understand the importance of a full loop from moment of choice to moment of choice, from the bedside to a diagnostic result and back. This implies the time from the moment when the need for diagnostics becomes clear, to the time when it can be acted upon based on its results. We call this time to action which is indicated by a red arrow in Figure 2.4.

#### **2.2.4 Multidisciplinary aspects of DSP and infection management**

It is essential to realise that the information needed to assess this time to action does not come only from microbiological laboratories. Communication and collaboration in the stewardship zone (Figures 2 and 3) are key and this applies to all specialities. But what would be the effect on the patient if microbiological diagnostics were not led by DSP when there is already good communication and cooperation in place? Would DSP no longer be necessary? Or is good cooperation equivalent to DSP?

DSP can significantly reduce the time to action by making proper use of each other's expertise to make optimal decisions for the patient. In practice, information from one diagnostic discipline can help to steer the diagnostic process of another diagnostic discipline. One reason for this is that during the diagnostic process of many disciplines, such as medical microbiology and imaging, an intrinsic amount of interpretation takes place. The clinical course is no less important here. We always need DSP, because together we try to act as optimally as possible in the interest of the patient, in which diagnosis is an important tool. DSP is not specific to medical microbiology, as demonstrated by the relevance of its collaboration with radiology in case 2. Nor is it specific to any other speciality. DSP is not intended as a reactive ad hoc solution but rather as a proactive, structural approach. DSP should be seen as guiding the entire diagnostic process, not only on the basis of antibiotics, but also on the basis of extensive imaging (such as for endocarditis), biomarkers (such as leukocytes and CRP, or procalcitonin for de-escalation of treatment), or by therapeutic drug monitoring (TDM) modelling the optimal dosage from the start of (empirical) treatment for individual patients and patient groups. One form of diagnostics is relevant to monitor trends, the other to directly answer a clinical question. This does not mean that one is less important than the other or that we should look at the value of an antibiogram differently from the value of a therapeutic drug monitoring. A pharmacist is also part of DSP.

As an example, in Dutch hospitals we are used to having a hospital pharmacist in house, providing clinical pharmaceutical services. Consultations are typically performed via e-mail, telephone, or an electronic prescription system. On the other hand, in countries such as the United Kingdom, these pharmacists work in infection management in the clinical (nursing) departments on a daily basis in collaboration with other specialists. This supports the most safe, appropriate,

and cost-effective antimicrobial treatment [15]. In addition, as mentioned earlier, the guidance of antimicrobial therapy by TDM is another important aspect. Hospital pharmacists can make suggestions on sample timing for TDM, inform about early prediction of attainable levels and dose adjustments to achieve adequate exposure and reduce toxicity as quickly as possible, and interpret results [16]. As a result, they are an integral part of the stewardship concept. We are convinced that the different stewardship terms and concepts form synergy for the best infection management [1,17]. Infection management has different aspects (such as ASP) and stewardship refers to guidance provided by focused experts [18].

Empirical antimicrobial therapy is a good example to illustrate how these aspects are linked. The working diagnosis (see also cases 1 and 2), based on an appropriate differential diagnosis, forms the basis for an appropriate empirical therapy that takes into account the most relevant pathogens, their anticipated susceptibility, the source of infection (taking into account the compartment), and underlying patient factors. Adequate initial diagnostic initiatives (such as deep focus puncture, see case 2) may simultaneously be therapeutic (such as surgical/interventional drainage for source control). Vice versa, the clinical course under therapy can be diagnostic in itself, for example, if diagnostics for the working diagnosis are correct and complete. Ultimately, the treatment of patients with complex infections almost always requires targeted treatment. This, in turn, requires adequate initial and ongoing diagnostics for optimal treatment. Figure 2.5 shows the decision moments and different specialisms that can be involved in this whole process.

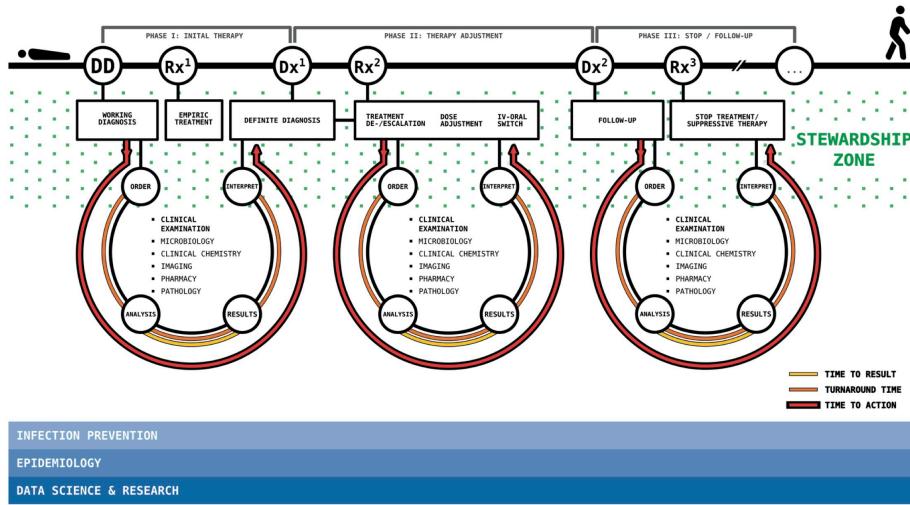


Figure 2.5: Stewardship in infection management.

## 2.3 Conclusion

The answer to the question from the title (Diagnostic stewardship - sentence or nonsense?) is: both. It is nonsense to debate terminology and the discussion about differences between diagnostic stewardship and infection management is only of semantic nature. Diagnostic stewardship makes sense in the concept discussed above. It can guide specialists (physician-microbiologist/medical-molecular microbiologists and experts from other fields, such as hospital pharmacists, radiologists, nuclear medicine, etc.) to the area of the stewardship zone of interaction and communication (Fig. 5), where they can bring in their expertise to complex clinical decision-making. Clinical information, including a patient's clinical development, is extremely important for correctly interpreting diagnostic results and steering the process. It can also help leading clinicians and other clinicians to understand the full potential (and limitations) of diagnostics and how important they are for evidence-based decision-making. We follow an integrated stewardship model that adds different perspectives (antimicrobial, infection prevention, and diagnostic stewardship - AID) to the ultimate goal of all stewardship intentions - the best quality care for the individual patient [1].

Stewardship consists largely of translation and communication during the decision-making process. Diagnostics are essential in this. But there is no need for a new name. Diagnostic stewardship as a name may be without added value and more and more use of stewardship-like terms could lead to confusion. The aim of all efforts and experts in infection management is the same: to improve quality of care and patient outcomes. We see with our own eyes how DSP guidelines are adhered to and realise how important it is that we continue to emphasise the often-underexposed diagnostic aspects of infection management. Multidisciplinary management based on diagnostics builds the basis for optimal outcomes for patients with infections.

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## **Chapter 3**

# **Introducing a New, Free, and Independent Method for Standardised, Reproducible and Reliable Analyses of Antimicrobial Resistance Data**

In preparation

(as of date of PhD defence: 25 August 2021)

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### **Abstract**

As the burden of antimicrobial resistance (AMR) is continuously increasing, reliable and reproducible data and data analysis are of utmost importance.

Conducting AMR data analysis is challenging since it requires (1) a thorough understanding of (clinical) epidemiology; (2) expertise in (clinical) microbiology and infectious diseases; (3) experience in microbiological data analysis; (4) availability of reference data, such as the biological taxonomy of microorganisms and defined daily doses (DDD) for antimicrobials; and (5) availability of (inter-)national guidelines and software methods to apply them. Furthermore, data stored in laboratory information systems lack the right structure, (inter-)national guidelines for interpreting raw laboratory test results cannot be easily applied, and scientifically reliable reference data about microorganisms and antimicrobial agents are not readily available. To fill this gap, we developed a free, independent, and open-source software solution to cover all those aspects of working with AMR data. The AMR package for R enables AMR data analysis for research and clinical workflows alike. Through an online survey package users reported more reproducibility of analysis results (83%), more reliable outcomes of AMR analyses (72%), and new or improved insight into AMR patterns (61%). The AMR package was also used to support clinical decision-making (44%) and for clinical research (28%). Our first insights into the usage and the usability of the AMR package confirm that this package is fulfilling its intended aim, as regional, national, and international organisations already use the package to support clinical decision-making in infection management. The flexible open-source design also enables rapid integration of updated guidelines (e.g., new EUCAST breakpoints) and setting-specific adaptations are encouraged. Together, the AMR package for R can thus empower any specialist in the field working with AMR data by providing a comprehensive toolbox of solutions for AMR data analyses.

### 3.1 Background

As the burden of antimicrobial resistance (AMR) is continuously increasing, surveillance programs with reliable and reproducible data and data analysis methods are of utmost importance for controlling and streamlining efforts to curb AMR [1,2]. To guide these efforts and to support clinical decision-making and infection-control interventions, AMR data analysis has to be conducted in a clinically and epidemiologically sensible way [3]. Conducting AMR data analysis is challenging since it requires (1) a thorough understanding of (clinical) epidemiology; (2) expertise in (clinical) microbiology and infectious diseases; (3) experience in microbiological data analysis; (4) availability of reference data, such as the biological taxonomy of microorganisms and defined daily doses (DDD) for antimicrobials; and (5) availability of (inter-)national guidelines and software methods to apply them.

Moreover, AMR data analysis is often also hindered by three key aspects. Firstly, data stored in microbiological laboratory information systems (LIS) are typically not readily suitable for (epidemiological) data analyses. LIS were initially designed to fit result registration and billing purposes rather than AMR

data analysis. Consequently, fundamental requirements for (epidemiological) data analyses are often lacking, such as isolate selection criteria, phenotypic determination of (multi-)drug resistance, and the ability to extract data for analysis in an automated, structured, fast, and reliable way. Moreover, data analyses that require data from multiple LIS sources (e.g., in multi-centre studies) face major barriers in data aggregation which, to the best of our knowledge, cannot be solved by currently available commercial software solutions. Besides, as applications of artificial intelligence are expected of being increasingly developed in the coming years, also in clinical microbiology, microbiological data technologies and structures need to become compatible for these future applications.

Secondly, AMR data analysis depends on (inter-)national standards and guidelines for the interpretation of raw laboratory measurements and the reporting of AMR results. In Europe, guidelines from the European Committee on Antimicrobial Susceptibility Testing (EUCAST) are the predominantly implemented set of rules in clinical microbiological laboratories [4,5]. LIS need to be well-maintained to be able to integrate continuous guideline updates. In our experience, this maintenance can often not be guaranteed and depends on the availability of local or external software support services. This is further hindered by the current distribution of manually formatted guidelines in Microsoft Excel and Portable Document Format (PDF) formats that are not often readily machine-readable. LIS maintainers, in collaboration with clinical staff, are therefore forced to manually implement updated guidelines which can be time-consuming and error-prone.

Thirdly, reliable AMR data analysis depends on taxonomic reference data to interpret raw LIS data using AMR interpretation guidelines, such as EUCAST Expert Rules and EUCAST Clinical Breakpoints [5,6]. Unfortunately, typical LIS contain local, static taxonomic data. We found that these data are often poorly maintained. We collected the taxonomic names of bacteria used in clinical reports from seven different public health institutions in the Netherlands which cover microbiological diagnostics in hospitals and primary care for 15% of the total Dutch population. The taxonomic names were compared to publicly available and authoritative reference databases; the Catalogue of Life and the List of Prokaryotic names with Standing in Nomenclature (LPSN, previously known as the Deutsche Sammlung von Mikroorganismen und Zellkulturen, DSMZ) [7,8]. We found that all participating institutions reported taxonomic names in clinical reports that did not match current taxonomic standards according to reference databases. For example, *Enterobacter aerogenes* and *Enterobacter massiliensis* were renamed *Klebsiella aerogenes* and *Metakosakonia massiliensis* respectively in 2017 [9,10]. LIS that are not kept up to date are consequently not entirely compatible with recent interpretation guidelines. Given that AMR guidelines are strongly based on the microbial taxonomy (some rules only apply to a specific genus, other rules apply to a specific family) it is crucial that this information is correct and kept up to date. In the studied institutions, the lag between the reported taxonomic names and the taxonomic standard was up to 41 years as

of March 2021.

## 3.2 Standardising AMR data analysis

Previously, no dedicated software solution was available to address all aforementioned aspects. To fill this gap, we developed a free, independent, and open-source software solution to cover all those aspects of working with AMR data. The AMR package for R [11] provides functionalities that enable standardised and reproducible workflows from any raw LIS data to results ready to publish, for research and clinical workflows alike. The AMR package for R was developed with a team of contributors from 12 public health organisations in seven countries aiming to be used in any research or clinical setting where (epidemiological) data analysis of microorganisms, AMR, or antimicrobial agents is required. It is independent of any other software solution and was designed to work in any setting, including those with limited computational and financial resources.

With this AMR package, we aimed at providing: (1) tools to simplify AMR data cleaning, transformation, and analysis; (2) methods to easily incorporate (inter)national guidelines; and (3) scientifically reliable reference data, including the aforementioned aspects. The AMR package enables standardised and reproducible AMR data analysis with the application of evidence-based rules (e.g., EUCAST expert rules for intrinsic resistance), the selection of first isolates, the translation of various codes for microorganisms and antimicrobial agents, determination of (multi-)drug-resistant microorganisms, and the calculation of antimicrobial resistance rates, prevalence, and future trends. The AMR package supports all EUCAST MIC/disk diffusion interpretation guidelines from 2011 until 2021 and EUCAST Expert rules versions 3.1 (2016) and 3.2 (2020) [12,13]. In addition, the AMR package supports all CLSI MIC/disk diffusion interpretation guidelines from 2011 until 2019 (non-veterinary only). For all mentioned guidelines, files readable for LIS are provided for easy implementation.

As of 30 April 2021, the AMR package for R has been downloaded from 162 countries since its first release in early 2018 (Figure 3.1), according to data from a popular public repository where users can download R packages. After 19 releases, the median number of downloads per release is 2,548 (range: 269-5,050).

A technical validation of the AMR package has been accepted for publication [11]. Additionally, it has been clinically and epidemiologically validated in a tertiary care hospital and across seven clinical microbiology laboratories in the Netherlands [Berends *et al.*, unpublished, see chapter 6 and 7 of this thesis]. Moreover, the AMR package has already been used in several scientific publications that focused on different aspects in the field of AMR [14-17].



Figure 3.1: Countries (grey, n = 162) with registered downloads of the AMR package for R between March 2018 and April 2021. Sources: cran.rstudio.org and cloud.r-project.org.

### 3.3 Comparison with existing software methods

Popular statistical software such as SPSS, Stata and SAS, focus on a broad implementation of statistical functions but are proprietary software, disallowing users to freely use, modify, or share the software. This also prohibits extending the software by unaffiliated developers. Since R is free, open software and extendible, users and developers can contribute to the software, to which end the AMR package is a practical example.

Other free software alternatives for AMR data analysis exist, for example WHONET, a free microbiology laboratory database software supported by the WHO [18]. WHONET allows manual data entry from LIS reports and provides AMR interpretation using recent CLSI and EUCAST guidelines with a particular focus on AMR surveillance. Results from WHONET can also be shared to surveillance programs such as the European Antimicrobial Resistance Surveillance Network (EARS-Net) and the WHO Global Antimicrobial Resistance Surveillance System (GLASS). Yet, the latest release, WHONET 2020, does not provide tools for cleaning and transforming data and relies on outdated EUCAST guidelines. Furthermore, we found a lag between the included taxonomic database and the current taxonomic standard of up to 59 years (median 7 years). Another alternative of a free software program is Epi Info which is provided by the United States Centers for Disease Control and Prevention (CDC) and aims at public health practitioners and researchers [19]. While Epi Info provides statistical and epidemiological methods for analysing data, it does not offer tools nor reference data for working with AMR test results or antimicrobial drugs, thus, ruling out the option for dedicated AMR data analysis. With the AMR package for R, an open and dedicated software solution is available that covers all aspects of working with AMR data.

### 3.4 User feedback

In July 2020, we published a survey on the website created for this package (<https://msberends.github.io/AMR>) to seek voluntary feedback from package users about user backgrounds and usage of the AMR package. Until December 2020, 18 participants completed the survey. Participants have used the AMR package in Australia, Colombia, Egypt, France, Germany, Haiti, India, Mali, Mexico, the Netherlands, Nigeria, Philippines, Spain, Sweden, and the United Kingdom.

Participants were asked to rate their experience in the statistical programming language R and in using the AMR package on a scale from 1 (not experienced/useful) to 10 (very experienced/useful). The overall experience in R was reported with a median of 7 (range: 4-9), whereas suitability for AMR analyses using the AMR package was rated with a median of 9 (range: 6-9). The participants rated the usefulness of the AMR package for their work with a median of 9 (range: 5-9). The convenience of the included software functions was rated with a median of 8 (range: 6-9) and the documentation of the AMR package was rated with a median of 8.5 (range: 7-10). Of all participants, 83% reported more reproducibility of analysis results and, 72% reported more reliable outcomes of AMR analyses (Figure 3.2). Notably, 61% reported new or improved insight into AMR for their institution or region. The AMR package was also used to support clinical decision-making (44%) and for clinical research (28%). Furthermore, 66% reported a faster and streamlined analysis workflow and 39% reported improved communicating analysis results. In 33%, participants started using R more often because of the capabilities that the AMR package provides.

Aside from AMR data analysis, most participants (78%) used the AMR package as a reference for the taxonomy of microorganisms. It was also regularly used for interpreting raw MIC and disk diffusion values (56%) and applying EUCAST expert rules (67%). This is in line with the original aims of the AMR package development.

### 3.5 Conclusion

AMR data analysis is dependent on (inter-)national guidelines and reliable (reference) data on the one hand but constrained by diverse and often inadequate data analysis tools and poor data quality on the other. We aimed to address these dependencies and constraints by introducing the AMR package for R for standardised and reproducible AMR data analyses. Our first insights into the usage and the usability of the AMR package confirm that this package is fulfilling its intended aim. Regional, national, and international organisations already use the AMR package to support clinical decision-making in infection management by gaining new or improved insights into resistance levels. We invite others to make use of our open-source approach and adapt it to their needs.

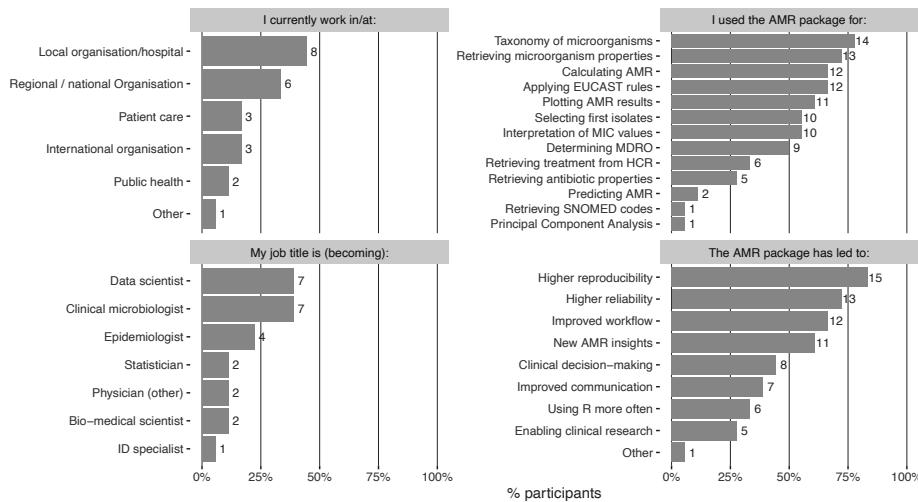


Figure 3.2: The outcome of the survey amongst 18 participants. MIC: minimal inhibitory concentration, MDRO: multidrug-resistant organism, SNOMED: Systematised Nomenclature of Medicine.

The advantages of sharing open-source software such as the AMR package allow for a collaborative, transparent use and further development that can lead to more standardised analysis processes for AMR data. The flexible open-source design also enables rapid integration of updated guidelines (e.g., new EUCAST breakpoints), and setting-specific adaptations are encouraged. Together, the AMR package for R can thus empower any specialist in the field working with AMR data by providing a comprehensive toolbox of solutions for AMR data analysis.

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## Chapter 4

# AMR - An R Package for Working with Antimicrobial Resistance Data

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## Abstract

Antimicrobial resistance is an increasing threat to global health. Evidence for this trend is generated in microbiological laboratories through testing microorganisms for resistance against antimicrobial agents. International standards and guidelines are in place for this process as well as for reporting data on (international) levels. However, there is a gap in the availability of standardised and reproducible tools for working with laboratory data to produce the required reports. It is known that extensive efforts in data cleaning and validation are

required when working with data from laboratory information systems. Furthermore, the global spread and relevance of antimicrobial resistance demands to incorporate international reference data in the analysis process. In this paper, we introduce the **AMR** package for R that aims at closing this gap by providing tools to simplify antimicrobial resistance data cleaning and analysis, while incorporating international guidelines and scientifically reliable reference data. The **AMR** package enables standardised and reproducible antimicrobial resistance analyses, including the application of evidence-based rules, determination of first isolates, translation of various codes for microorganisms and antimicrobial agents, determination of (multi-drug) resistant microorganisms, and calculation of antimicrobial resistance, prevalence and future trends. The **AMR** package works independently of any laboratory information system and provides several functions to integrate into international workflows (e.g., WHONET software provided by the World Health Organization).

## 4.1 Introduction

Antimicrobial resistance is a global health problem and of great concern for human medicine, veterinary medicine, and the environment alike. It is associated with significant burdens to both patients and health care systems. Current estimates show the immense dimensions we are already facing, such as claiming at least 50,000 lives due to antimicrobial resistance each year across Europe and the US alone [1]. Although estimates for the burden through antimicrobial resistance and their predictions are disputed [2] the rising trend is undeniable [3], thus calling for worldwide efforts on tackling this problem.

Surveillance programs and reliable data are key for controlling and streamlining these efforts. Surveillance data of antimicrobial resistance at higher levels (national or international) usually comprise aggregated numbers. The basis of this information is generated and stored at local microbiological laboratories where isolated microorganisms are tested for their susceptibility to a whole range of antimicrobial agents. The efficacy of these agents against microorganisms is nowadays interpreted as follows [4]:

- R (“resistant”) - there is a high likelihood of therapeutic failure;
- S (“susceptible, standard dosing regimen”) - there is a high likelihood of therapeutic success using a standard dosing regimen of an antimicrobial agent;
- I (“susceptible, increased exposure”) - there is a high likelihood of therapeutic success, but only when exposure to an antimicrobial agent is increased by adjusting the dosing regimen or its concentration at the site of infection.

Generally, antimicrobial resistance is defined as the proportion of resistant microorganisms (R) among all tested microorganisms of the same species (R + S + I). Today, the two major guideline institutes to define the international

standards on antimicrobial resistance are the European Committee on Antimicrobial Susceptibility Testing (EUCAST) [5] and the Clinical and Laboratory Standards Institute (CLSI) [6]. The guidelines from these two institutes are adopted by 94% of all countries reporting antimicrobial resistance to the WHO [7].

Although these standardised guidelines are in place on the laboratory level for the data generation process, stored data in laboratory information systems are often not yet suitable for data analysis. Laboratory information systems are often designed to fit billing purposes rather than epidemiological data analysis. Furthermore, (inter-)national surveillance is hindered by inadequate standardisation of epidemiological definitions, different types of samples and data collection, settings included, microbiological testing methods (including susceptibility testing), and data sharing policies [8]. The necessity of accurate data analysis in the field of antimicrobial resistance has just recently been further underlined [9]. Antimicrobial resistance analyses require a thorough understanding of microbiological tests and their results, the biological taxonomy of microorganisms, the clinical and epidemiological relevance of the results, their pharmaceutical implications, and (inter-)national standards and guidelines for working with and reporting antimicrobial resistance.

Here, we describe the **AMR** package for R [10], which has been developed to standardise clean and reproducible antimicrobial resistance data analyses using international standardised recommendations [5,6] while incorporating scientifically reliable reference data about valid laboratory outcome, antimicrobial agents, and the complete biological taxonomy of microorganisms. The **AMR** package provides solutions and support for these aspects while being independent of underlying laboratory information systems, thereby democratising the analysis process. Developed in R and available on the ComprehensiveR Archive Network (CRAN) since February 22nd 2018 [11], the **AMR** package enables reproducible workflows as described in other fields, such as environmental science [12]. The **AMR** package provides a new technical instrument to aid in curbing the global threat of antimicrobial resistance. Furthermore, local, and regional data in the laboratories can now become relevant in any setting for public health.

While no other packagesR package with the purpose of dealing with antimicrobial resistance data are available on CRAN or Bioconductor, the **AMR** package may be integrated in workflows of related packages. For example, theR Epidemics Consortium (RECON) provides high-quality packages for data analysis in infectious disease outbreaks or epidemics (for example incidence and epi-contacts) [13,14]. In addition, on the laboratory side the antibioticR package provides approaches to work with disc diffusion zone diameter and minimum inhibitory concentration data from environment samples [15]. We aim at providing a comprehensive and standardised toolbox for antimicrobial resistance data processing and analysis, with a focus on microbiological, clinical, and epidemiological purposes that was yet missing.

The following sections describe the functionality of the **AMR** package according to

its core functionalities for transforming, enhancing, and analysing antimicrobial resistance data using scientifically reliable reference data.

## 4.2 Antimicrobial resistance data

Microbiological tests can be performed on different specimens, such as blood or urine samples or nasal swabs. After arrival at the microbiological laboratory, the specimens are traditionally cultured on specific media, such as blood agar. If a microorganism can be isolated from these media, it is tested against several antimicrobial agents. Based on the minimal inhibitory concentration (MIC) of the respective agent and interpretation guidelines, such as guidelines by EUCAST [5] and CLSI [6], test results are reported as “resistant” (R), “susceptible” (S) or “susceptible, increased exposure” (I). A typical data structure is illustrated in Table 1 [5].

Table 1. Example of an antimicrobial resistance report.

patient	date	test_no	specimen	mo	PEN	AMC	CIP
000001	2019-03-08	100	blood	esccol	R	I	S
000001	2019-03-09	101	blood	esccol	R	I	S
000002	2019-03-08	102	blood	staaur	R	S	-
000003	2019-03-08	103	urine	pseae	R	R	R

R = resistant, S = susceptible, I = susceptible, increased exposure, mo = microorganism, PEN = penicillin, AMC = amoxicillin/clavulanic acid, CIP = ciprofloxacin.

Table 2. Example of an antimicrobial resistance report.

patient	date	test_no	specimen	mo	PEN	AMC	CIP
1	2019-03-08	100	blood	esccol	R	I	S
1	2019-03-09	101	blood	esccol	R	I	S
2	2019-03-08	102	blood	StaAur	>8 (R)*	<0.01 (S)*	.
00003	2019-03-08	103	urine	P. aeru.	R	S**	S

R = resistant, S = susceptible, I = susceptible, increased exposure, mo = microorganism, PEN = penicillin, AMC = amoxicillin/clavulanic acid, CIP = ciprofloxacin.

\* Mixed reporting of minimal inhibitory concentration (MIC) and susceptibility interpretation of MIC value

\*\* False reporting: *Pseudomonas aeruginosa* (mo = P. aeru.) is intrinsically resistant to amoxicillin/clavulanic acid (AMC)

The AMR package aims at providing a standardised and automated way of cleaning, transforming, and enhancing these typical data structures (Table 1 and 2), independent of the underlying data source. Processed data would be similar to Table 3 that highlights several package functionalities in the sections below.

Table 3. Enhanced antimicrobial resistance report example.

patient	date	test_no	specimen	mo	PEN	AMC	CIP	first_isolate	name	gram_stain
000001	2019-03-08	100	blood	B_ESCHR_COLI	R	I	S	TRUE	Escherichia coli	Gram-negative
000001	2019-03-09	101	blood	B_ESCHR_COLI	R	I	S	FALSE	Escherichia coli	Gram-negative
000002	2019-03-08	102	blood	B_STPHY_AURS	R	S	-	TRUE	Staphylococcus aureus	Gram-positive
000003	2019-03-08	103	urine	B_PSDMN_AERG	R	R	R	TRUE	Pseudomonas aeruginosa	Gram-negative

## 4.3 Antimicrobial resistance data transformation

### 4.3.1 Working with taxonomically valid microorganism names

Coercing is a computational process of forcing output based on an input. For microorganism names, coercing user input to taxonomically valid microorganism names is crucial to ensure correct interpretation and to enable grouping based on taxonomic properties. To this end, the **AMR** package includes all microbial entries from The Catalogue of Life (<http://www.catalogueoflife.org>), the most comprehensive and authoritative global index of species currently available [16]. It holds essential information on the names, relationships, and distributions of more than 1.9 million species. The integration of it into the **AMR** package is described in Appendix A.

The **as.mo()** function makes use of this underlying data to transform a vector of characters to a new class ‘‘mo’’ of taxonomically valid microorganism name. The resulting values are microbial IDs, which are human-readable for the trained eye and contain information about the taxonomic kingdom, genus, species, and subspecies (Figure 1).

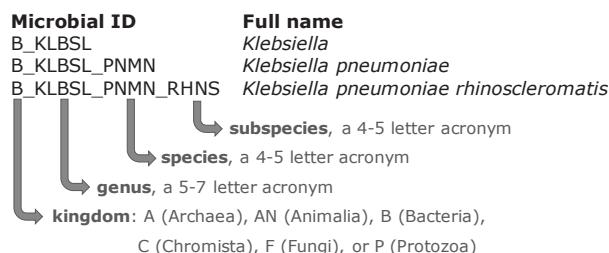


Figure 4.1: The structure of a typical microbial ID as used in the ‘AMR’ package. An ID consists of two to four elements, separated by an underscore. The first element is the abbreviation of the taxonomic kingdom. The remaining elements consist of abbreviations of the lowest taxonomic levels of every microorganism: genus, species (if available) and subspecies (if available). Abbreviations used for the microbial IDs of microorganism names were created using the base ‘R’ function ‘abbreviate()’.

The `as.mo()` function compares the user input with taxonomically valid microorganism names, rates the matching with a score and returns results based on the highest score. This matching score ( $m$ ), ranging from 0 to 1, is calculated using the following equation:

$$m_{(x,n)} = \frac{l_n - 0.5 \cdot \min\{l_n, \text{lev}(x, n)\}}{l_n \cdot p_n \cdot k_n}$$

where:

- $x$  is the user input;
- $n$  is a taxonomic name (genus, species, and subspecies);
- $l_n$  is the length of  $n$ ;
- $\text{lev}$  is the Levenshtein distance function [17], which counts any insertion, deletion and substitution as 1 that is needed to change  $x$  into  $n$ ;
- $p_n$  is the human pathogenic prevalence group of  $n$ , as described below;
- $k_n$  is the taxonomic kingdom of  $n$ , set as Bacteria = 1, Fungi = 2, Protozoa = 3, Archaea = 4, others = 5.

The grouping into human pathogenic prevalence ( $p$ ) is based on experience from several microbiological laboratories in the Netherlands in conjunction with international reports on pathogen prevalence [7,18,19]. **Group 1** (most prevalent microorganisms) consists of all microorganisms where the taxonomic class is Gammaproteobacteria or where the taxonomic genus is *Enterococcus*, *Staphylococcus* or *Streptococcus*. This group consequently contains all common Gram-negative bacteria, such as *Pseudomonas* and *Legionella* and all species within the order Enterobacterales. **Group 2** consists of all microorganisms where the taxonomic phylum is Proteobacteria, Firmicutes, Actinobacteria or Sarcomastigophora, or where the taxonomic genus is *Absidia*, *Acremonium*, *Actinotignum*, *Alternaria*, *Anaerosalibacter*, *Apophysomyces*, *Arachnia*, *Aspergillus*, *Aureobacterium*, *Aureobasidium*, *Bacteroides*, *Basidiobolus*, *Beauveria*, *Blastocystis*, *Branhamella*, *Calymmatobacterium*, *Candida*, *Capnocytophaga*, *Catabacter*, *Chaetomium*, *Chryseobacterium*, *Chrysosomonas*, *Chrysonilia*, *Cladophialophora*, *Cladosporium*, *Conidiobolus*, *Cryptococcus*, *Curvularia*, *Exophiala*, *Exserohilum*, *Flavobacterium*, *Fonsecaea*, *Fusarium*, *Fusobacterium*, *Hendersonula*, *Hypomyces*, *Koserella*, *Lelliottia*, *Leptosphaeria*, *Leptotrichia*, *Malassezia*, *Malbranchea*, *Mortierella*, *Mucor*, *Mycocentrospora*, *Mycoplasma*, *Nectria*, *Ochroconis*, *Oidiodendron*, *Phoma*, *Piedraia*, *Pithomyces*, *Pityrosporum*, *Prevotella*, *Pseudallescheria*, *Rhizomucor*, *Rhizophorus*, *Rhodotorula*, *Scolecobasidium*, *Scopulariopsis*, *Scytalidium*, *Sporobolomyces*, *Stachybotrys*, *Stomatococcus*, *Treponema*, *Trichoderma*, *Trichophyton*, *Trichosporon*, *Trirachium* or *Ureaplasma*. **Group 3** consists of all other microorganisms.

This will lead to the effect that e.g., "E. coli" will return the microbial ID of *Escherichia coli* ( $m = 0.688$ , a highly prevalent microorganism found in humans) and not *Entamoeba coli* ( $m = 0.079$ , a less prevalent microorganism

in humans), although the latter would alphabetically come first. The matching score function is for users available as `mo_matching_score()`.

If any coercion rules are applied, a warning is printed to the console and scores can be reviewed by calling `mo_uncertainties()`, that prints all other matches with their matching scores. Users can furthermore control the coercion rules by setting the `allow_uncertain` argument in the `as.mo()` function. The following values or levels can be used:

- 0: no additional rules are applied;
- 1: allow previously accepted (but now invalid) taxonomic names and minor spelling errors;
- 2: allow all of 1, strip values between brackets, inverse the words of the input, strip off text elements from the end keeping at least two elements;
- 3: allow all of level 1 and 2, strip off text elements from the end, allow any part of a taxonomic name;
- TRUE (default): equivalent to 2;
- FALSE: equivalent to 0.

To support organisation specific microbial IDs, users can specify a custom reference ‘`data.frame`’, by using `as.mo(..., reference_df = ...)`. This process can also be automated by users with the `set_mo_source()` function.

#### 4.3.1.1 Properties of microorganisms

The package contains functions to return a specific (taxonomic) property of a microorganism from the `microorganisms` data set (see Appendix A). Functions that start with `mo_*` can be used to retrieve the most recently defined taxonomic properties of any microorganism quickly and conveniently. These functions rely on the `as.mo()` function internally: `mo_name()`, `mo_fullname()`, `mo_shortname()`, `mo_subspecies()`, `mo_species()`, `mo_genus()`, `mo_family()`, `mo_order()`, `mo_class()`, `mo_phylum()`, `mo_kingdom()`, `mo_type()`, `mo_gramstain()`, `mo_ref()`, `mo_authors()`, `mo_year()`, `mo_rank()`, `mo_taxonomy()`, `mo_synonyms()`, `mo_info()` and `mo_url()`. Determination of the Gram stain, by using `mo_gramstain()`, is based on the taxonomic subkingdom and phylum. According to Cavalier-Smith [20], who defined the subkingdoms Negibacteria and Posibacteria, only the following phyla are Posibacteria: Actinobacteria, Chloroflexi, Firmicutes and Tenericutes. Bacteria from these phyla are considered Gram-positive - all other bacteria are considered Gram-negative. Gram stains are only relevant for species within the kingdom of Bacteria. For species outside this kingdom, `mo_gramstain()` will return NA.

#### 4.3.2 Working with antimicrobial names or codes

The AMR package includes the `antibiotics` data set, which comprises common laboratory information system codes, official names, ATC (Anatomical Therapeutic Chemical) codes, defined daily doses (DDD) and more than 5,000 trade

names of 456 antimicrobial agents (see Appendix A). The ATC code system and the reference list for DDDs have been developed and made available by the World Health Organization Collaborating Centre for Drug Statistics Methodology (WHOCC) to standardise pharmaceutical classifications [21]. All agents in the `antibiotics` data set have a unique antimicrobial ID, which is based on abbreviations used by the European Antimicrobial Resistance Surveillance Network (EARS-Net), the largest publicly funded system for antimicrobial resistance surveillance in Europe [22]. Furthermore, the `AMR` package includes the antivirals data set containing antiviral agents, which is also described in Appendix A.

#### 4.3.2.1 Properties of antimicrobial agents

It is a common task in microbiological data analyses (and other clinical or epidemiological fields) to work with different antimicrobial agents. The `AMR` package provides several functions to translate inputs such as ATC codes, abbreviations, or names in any direction. Using `as.ab()`, any input will be transformed to an antimicrobial ID of class ‘`ab`’. Helper functions are available to get specific properties of antimicrobial IDs, such as `ab_name()` for getting the official name, `ab_atc()` for the ATC code, or `ab_cid()` for the CID (Compound ID) used by PubChem [23]. Trade names can be also used as input. For example, the input values “Amoxil”, “dispermox”, “amox” and “J01CA04” all return the ID of amoxicillin (AMX):

```
as.ab("Amoxicillin")
#> Class <ab>
#> [1] AMX

as.ab(c("Amoxil", "dispermox", "amox", "J01CA04"))
#> Class <ab>
#> [1] AMX AMX AMX AMX

ab_name("Amoxicillin")
#> [1] "Amoxicillin"

ab_atc("amox")
#> [1] "J01CA04"

ab_name("J01CA04")
#> [1] "Amoxicillin"
```

If more than one antimicrobial agent is found in the input string, a warning with the additional findings is printed to the console.

#### 4.3.2.2 Filtering data based on classes of antimicrobial agents

The application of the ATC classification system also enables grouping of antimicrobial agents for data analyses. Data sets with microbial isolates can be filtered on isolates with specific results for tested antimicrobial agents in a specific antimicrobial class. For example, using `filter_carbapenems(result = "R")` returns data of all isolates with tested resistance to any of the 14 available antimicrobial agents in the group of carbapenems according to the `antibiotics` data set.

#### 4.3.3 Working with antimicrobial susceptibility test results

Minimal inhibitory concentrations (MIC) are susceptibility test results measured by microbiological laboratory equipment to determine at which minimum antimicrobial drug concentration 99.9% of a microorganism is inhibited in growth. These concentrations are often capped at a minimum and maximum, for example 0.02 µg/ml and 32 µg/ml, respectively. The ‘`mic`’ class, an ordered ‘`factor`’ containing valid MIC values, keeps these operators while still ordering all possible outcomes correctly so that e.g., “`<=0.02`” will be considered lower than “`0.04`”.

Another susceptibility testing method is the use of drug diffusion disks, which are small tablets containing a specified concentration of an antimicrobial agent. These disks are applied onto a solid growth medium or a specific agar plate. After 24 hours of incubation, the diameter of the growth inhibition around a disk can be measured in millimetres with a ruler. The ‘`disk`’ class can be used to clean these kinds of measurements, since they should always be valid numeric values between 6 and 50. The supported minima and maxima of valid values for both classes, ‘`mic`’ and ‘`disk`’, are displayed in Table 4.

Table 4. Antimicrobial susceptibility test classes.

Class	Minimum	Maximum	Unit
‘ <code>mic</code> ’	≤0.001	≥1024	µg/ml
‘ <code>disk</code> ’	≤6	≥50	mm

The higher the MIC or the smaller the growth inhibition diameter, the more active substance of an antimicrobial agent is needed to inhibit cell growth, i.e. the higher the antimicrobial resistance against the tested antimicrobial agent. At high MICs and small diameters, guidelines interpret the microorganism as “resistant” (R) to the tested antimicrobial agent. At low MICs and wide diameters, guidelines interpret the microorganism as “susceptible” (S) to the tested antimicrobial agent. In between, the microorganism is classified as “susceptible, increased exposure” (I). For these three interpretations the ‘`rsi`’ class has been developed. When using `as.rsi()` on MIC values (of class ‘`mic`’) or disk diffusion diameters (of class ‘`disk`’), the values will be interpreted according to the

guidelines from the CLSI or EUCAST (all guidelines between 2011 and 2020 are included in the AMR package) [24,25]. Guidelines can be changed by setting the guidelines argument.

```
# Low MIC value
as.rsi(as.mic(2), "E. coli", "ampicillin", guideline = "EUCAST 2020")
#> Class <rsi>
#> [1] S

# High MIC value
as.rsi(as.mic(32), "E. coli", "ampicillin", guideline = "EUCAST 2020")
#> Class <rsi>
#> [1] R
```

When using the `as.rsi()` function on existing antimicrobial interpretations, it tries to coerce the input to the values “R”, “S” or “I”. These values can in turn be used to calculate the proportion of antimicrobial resistance.

#### 4.3.4 Interpretative rules by EUCAST

Next to supplying guidelines to interpret raw MIC values, EUCAST has developed a set of expert rules to assist clinical microbiologists in the interpretation and reporting of antimicrobial susceptibility tests [5]. The rules comprise assistance on intrinsic resistance, exceptional phenotypes, and interpretive rules. The AMR package covers intrinsic resistant and interpretive rules for data transformation and standardisation purposes. The first prevents false susceptibility reporting by providing a list of organisms with known intrinsic resistance to specific antimicrobial agents (e.g., cephalosporin resistance of all enterococci). Interpretive rules apply inference from established resistance mechanisms [26-29]. Both groups of rules are based on classic IF THEN statements (e.g., IF Enterococcus spp. resistant to ampicillin THEN also report as resistant to imipenem). Some rules provide assistance for further actions when certain resistance has been detected, i.e., performing additional testing of the isolated microorganism. The AMR package function `eucast_rules()` can apply all EUCAST rules that do not rely on additional clinical information, such as additional information on patients' diagnoses. Table 2 and 3 highlight the transformation for the reporting of AMX = S in patient\_id = 000003 to the correct report according to EUCAST rules of AMX = R. Of note, however, EUCAST rules overwrite original data to correct for the difference in how antimicrobial agents affect the tested microorganism in vitro (in the laboratory) and in vivo (in the human body). This requires users to closely collaborate with the data source provider to ensure correct versioning, backward compatibility, reproducibility, and taking into account specific local regulation for resistance reporting. Typical scenarios where changes to the original data points apply include in vitro test results indicating susceptibility when resistance in vivo is known. The changes are based on scientific consensus to ensure reliable high-quality reporting of antimicrobial susceptibility results. All changes to the data are printed to the console

and can also be reviewed in detail by setting the argument `eucast_rules(..., verbose = TRUE)`.

EUCAST rules are subject to regular updates which are implemented into the AMR package by the AMR maintenance team shortly after publication. The `eucast_rules()` function supports versioning of the rules. The arguments `version_breakpoints` and `version_expertrules` can be set to current or previous versions. By default, the `eucast_rules()` function uses the latest implemented version.

### 4.3.5 Working with defined daily doses (DDD)

DDDs are essential for standardising antimicrobial consumption analysis, for inter-institutional or international comparison. The official DDDs are published by the WHOCC [36]. Updates to the official publication are monitored by the AMR maintenance team and implemented in the `antibiotics` data set included in the AMR package. Other metrics exist such as the recommended daily dose (RDD) or the prescribed daily dose (PDD). However, DDDs are the only metric that is independent of a patient's disease and therapeutic choices and thus suitable for the AMR package.

Functions from the `atc_online_*`() family take any text as input that can be coerced with `as.ab()` (i.e., to class ‘ab’). Next, the functions access the WHOCC online registry [30] (internet connection required) and download the property defined in the arguments (e.g., `administration = "O"` or `administration = "P"` for oral or parenteral administration and `property = "ddd"` or `property = "groups"` to get DDD or the group of the selected antimicrobial defined by its ATC code).

```
atc_online_ddd("amoxicillin", administration = "O")
#> [1] 1.5

atc_online_groups("amoxicillin")
#> [1] "ANTIINFECTIVES FOR SYSTEMIC USE"
#> [2] "ANTIBACTERIALS FOR SYSTEMIC USE"
#> [3] "BETA-LACTAM ANTIBACTERIALS, PENICILLINS"
#> [4] "Penicillins with extended spectrum"
```

## 4.4 Enhancing antimicrobial resistance data

### 4.4.1 Determining first isolates

Determining antimicrobial resistance or susceptibility can be done for a single agent (mono- therapy) or multiple agents (combination therapy). The calculation of antimicrobial resistance statistics is dependent on two prerequisites: the data should only comprise the first isolates and a minimum required number of 30 isolates should be met for every stratum in further analysis [6].

An isolate is a microorganism strain cultivated on specified growth media in a laboratory, so its phenotype can be determined. First isolates are isolates of any species found first in a patient per episode, regardless of the body site or the type of specimen (such as blood or urine) [6]. The selection on first isolates (using function `first_isolate()`) is important to prevent selection bias, as it would lead to overestimated or underestimated resistance to an antimicrobial agent. For example, if a patient is admitted with a multi-drug resistant microorganism and that microorganism is found in five different blood cultures the following week, it would overestimate resistance if all isolates were to be included in the analysis.

The episode in days can be set with the argument `episode_days`, which defaults to 365 as suggested by the CLSI guideline [6].

#### 4.4.2 Determining multi-drug resistant organisms (MDRO)

Definitions of multi-drug resistant organisms (MDRO) are regulated by national and international expert groups and differ between nations. The `AMR` package provides the functionality to quickly identify MDROs in a data set using the `mdro()` function. Guidelines can be set with the argument `guideline`. At default, it applies the guideline as proposed by Magiorakos *et al.* [31]. Their work describes the definitions for bacteria being ‘MDR’ (multi-drug-resistant), ‘XDR’ (extensively drug-resistant) or ‘PDR’ (pan-drug-resistant). These definitions are widely adopted [32] and known in the field of medical microbiology.

Other guidelines currently supported are the international EUCAST guideline (`guideline = “EUCAST”` [33]), the international WHO guideline on the management of drug-resistant tuberculosis (`guideline = “TB”` [34]), and the national guidelines of The Netherlands (`guideline = “NL”` [35]), and Germany (`guideline = “DE”` [36]).

Some guidelines require a minimum availability of tested antimicrobial agents per isolate. This is needed to prevent false-negatives, since no reliable determination can be performed on only a few test results. This required minimum defaults to 50%, but can be set by the user with the `pct_minimum_classes`. Isolates that do not meet this requirement will be skipped for determination and will return `NA` (not applicable), with an informative warning printed to the console.

The rules are applied per row of the data. The `mdro()` function automatically identifies the variables containing the microorganism codes and antimicrobial agents based on the `guess_ab_col()` function. Following the guideline set by the user, it analyses the specific antimicrobial resistance of a microorganism and flags that microorganism accordingly. The outcome is demonstrated in Table 5, where the first row is an MDRO according to the Dutch guidelines [35].

Table 5. Example of a multi-drug resistant organism (MDRO) in a data set

identified by applying Dutch guidelines.

mo	AMC	GEN	TOB	CIP	MFX	MDRO
B_ESCHR_COLI	S	R	R	R	R	Positive
B_ESCHR_COLI	R	S	R	R	S	Negative
B_ESCHR_COLI	S	S	S	R	S	Negative

mo = microorganism, AMC = amoxicillin/clavulanic acid, GEN = gentamicin, TOB = tobramycin, CIP = ciprofloxacin, MFX = moxifloxacin, MDRO = multi-drug resistant organism, B\_ESCHR\_COLI = microorganism code of *Escherichia coli*.

#### 4.4.2.1 Multi-drug resistant tuberculosis

Tuberculosis is a major threat to global health caused by *Mycobacterium tuberculosis* (MTB) and is one of the top ten causes of death worldwide [37]. Exceptional antimicrobial resistance in MTB is therefore of special interest. To this end, the international WHO guideline for the classification of drug resistance in MTB [34] is included in the AMR package. The `mdr_tb()` function is a convenient wrapper around `mdro(..., guideline = "TB")`, which returns an other ordered ‘factor’ than other `mdro()` functions. The output will contain the ‘factor’ levels ‘Negative’ < ‘Mono-resistant’ < ‘Poly-resistant’ < ‘Multi-drug-resistant’ < ‘Extensively drug-resistant’, following the WHO guideline.

## 4.5 Analysing antimicrobial resistance data

### 4.5.1 Calculation of antimicrobial resistance

The AMR package contains several functions for fast and simple resistance calculations of bacterial or fungal isolates. A minimum number of available isolates is needed for the reliability of the outcome. The CLSI guideline suggests a minimum of 30 available first isolates irrespective of the type of statistical analysis [6]. Therefore, this number is used as the default setting for any function in the package that calculates antimicrobial resistance or susceptibility, which can be changed with the `minimum` argument in all applicable functions.

#### 4.5.1.1 Counts

The AMR package relies on the concept of tidy data [38], although not strictly following its rules (one row per test rather than one row per observation). Function names to calculate the number of available isolates follow these general resistance interpretation standards with `count_S()`, `count_I()`, and `count_R()` respectively. Combinations of antimicrobial resistance interpretations can be counted with `count_SI()` and `count_IR()`. All these functions take a vector of interpretations of the class ‘rsi’ (as discussed above) or are internally transformed with `as.rsi()`. The returned value is the sum of the respective interpretation in the selected test column. All `count_*` functions

support quasi-quotation with pipes, grouped variables, and can be used with `dplyr::summarise()`<sup>[39]</sup>.

#### 4.5.1.2 Proportions

Calculation of antimicrobial resistance is carried out by counting the number of first resistant isolates (interpretation of “R”) and dividing it by the number of all first isolates, see Equation 2. This is implemented in the `proportion_R()` function. To calculate antimicrobial susceptibility, the number of susceptible first isolates (interpretation of “S” and “I”) has to be counted and divided by the number of all first isolates, which is implemented in the `proportion_SI()` function. For convenience, the `resistance()` function is an alias of the `proportion_R()` function, and the `susceptibility()` function is an alias of the `proportion_SI()` function.

The functions `proportion_R()`, `proportion_IR()`, `proportion_I()`, `proportion_SI()`, and `proportion_S()` follow the same logic as the `count_*` functions and all return a vector of class ‘double’ with a value between 0 and 1. The argument `minimum` defines the minimal allowed number of available (tested) isolates (default: `minimum = 30`). Any number below the set minimum will return `NA` with a warning.

For calculating the proportion ( $P$ ) of antimicrobial resistance or susceptibility to one antimicrobial agent, the following equation is used:

$$P_{(x,o)} = \frac{\sum_{i=1}^k [x_i \in o]}{\sum_{i=1}^k [x_i \in \{R, S, I\}]}$$

where  $P$  is the proportion of outcome  $o$  (that is either “R”, “S”, “I”, or a combination of two of them), where  $x$  is a character vector of length  $k$  only consisting of values “R”, “S”, or “I” and  $[x_i \in o]$  is the indicator function, returning 1 if the indicator function is true and 0 otherwise. The denominator must include the collection  $\{R, S, I\}$  so that ‘wrong’ elements in  $x$  (i.e., elements not being “R”, “S”, or “I”) will not be counted. Thus, the theoretical antimicrobial susceptibility of the vector  $x = \{S, S, I, R, R\}$  is:

$$P_{(x,o=\{S,I\})} = \frac{3}{5} = 0.6$$

For the proportion of empiric susceptibility ( $s$ ) for more than one antimicrobial agent, the calculation can be carried out in two ways (Table 6).

Table 6. Example calculation for determining empiric susceptibility (%SI) for more than one antimicrobial agent.

The first method is to count the total number of first isolates where at least one agent was tested as “S” or “I” and divide it by the number of first isolates tested

Antimicrobial agent		All isolates ( <code>only_all_tested = FALSE</code> )		Only isolates tested for both agents ( <code>only_all_tested = TRUE</code> )	
Agent A	Agent B	Include as numerator	Include as denominator	Include as numerator	Include as denominator
S or I	S or I	X	X	X	X
R	S or I	X	X	X	X
NA	S or I	X	X		
S or I	R	X	X	X	X
R	R		X		X
NA	R				
S or I	NA	X	X		
R	NA				
NA	NA				

R = resistant, S = susceptible, I = susceptible, increased exposure, NA = not available; not tested or missing.

where any of the agents was tested (Equation 4). This method will be used when setting `only_all_tested = FALSE` in the `susceptibility()` function:

$$s_{(x,y)} = \frac{\sum_{i=1}^k [x_i \in \{S, I\} \vee y_i \in \{S, I\}]}{\sum_{i=1}^k [x_i \in \{R, S, I\} \vee y_i \in \{R, S, I\}]}$$

where  $x$  is a character vector only consisting of values “R”, “S”, or “I” (i.e., ‘agent A’) and  $y$  is another character vector only consisting of values “R”, “S”, or “I” (i.e., ‘agent B’).

The second method is to count the total number of first isolates where at least one agent was tested as “S” or “I” and where all agents were tested divided by the number of first isolates tested where all of the agents were tested (Equation 5). This method will be used when setting `only_all_tested = TRUE` in the `susceptibility()` function:

$$s'_{(x,y)} = \frac{\sum_{i=1}^k [(x_i \in \{S, I\} \vee y_i \in \{S, I\}) \wedge x_i \in \{R, S, I\} \wedge y_i \in \{R, S, I\}]}{\sum_{i=1}^k [x_i \in \{R, S, I\} \wedge y_i \in \{R, S, I\}]}$$

Based on Equation 2, the overall resistance and susceptibility of antimicrobial agents like gentamicin (GEN) and amoxicillin (AMX) can be calculated using the following syntax. The `example_isolates` data set is an example data set included in the `AMR` package, see Appendix A. The `n_rsi()` function is analogous to the `n()` function of the `dplyr` package. It counts the number of available isolates, but only includes observations with valid antimicrobial results (i.e., “R”, “S”, or “I”):

```
library("dplyr")
example_isolates %>%
  summarise(r_gen = proportion_R(GEN),
            r_amx = proportion_R(AMX),
            n_gen = n_rsi(GEN),
            n_amx = n_rsi(AMX),
            n_total = n())
#>      r_gen      r_amx n_gen n_amx n_total
#> [1] 0.2458221 0.5955556 1855 1350   2000
```

This output reads: the resistance to gentamicin of all isolates in the `example_isolates` data set is  $P(x = \text{GEN}, o = \{R\}) = 24.6\%$ , based on 1855 out of 2000 available isolates. This means that the susceptibility is  $P(x = \text{GEN}, o = \{S, I\}) = 75.4\%$ . The susceptibility to amoxicillin is  $P(x = \text{AMX}, o = \{S, I\}) = 40.4\%$  based on 1350 isolates.

To calculate the effect of combination therapy, i.e., treating patients with multiple agents at the same time, all `proportion_*`() functions can handle multiple variables as arguments as defined in Equation 4 and 5. For example, to calculate the empiric susceptibility of a combination therapy comprising gentamicin (GEN) and amoxicillin (AMX):

```
example_isolates %>%
  summarise(si_gen_amx = proportion_SI(GEN, AMX),
            n_gen_amx = n_rsi(GEN, AMX),
            n_total = n())
#>      si_gen_amx n_gen_amx n_total
#> [1]    0.931843     1921    2000
```

This leads to the conclusion that combining gentamicin with amoxicillin would cover  $s(x = \text{GEN}, y = \text{AMX}) = 93.2\%$  based on 1921 out of 2000 available isolates, which is 17.8% more than when treating with gentamicin alone ( $P(x = \text{GEN}, o = \{S, I\}) = 75.4\%$ ). With these functions, exact calculations can be done to evaluate the empiric success of treating infections with one or more antimicrobial agents.

## 4.6 Design decisions

The AMR package follows the rationale of tidyverse packages as authored by Wickham *et al.* [40]. Most functions take a ‘`data.frame`’ or ‘`tibble`’ as input, support piping (%>%) operations, can work with quasi-quotations, and can be integrated into `dplyr` workflows, such as `mutate()` to create new variables and `group_by()` to group by variables. Although the AMR package integrates well into tidyverse workflows, it can also be used with base Ronly. To this extent, the AMR package was developed to be independent of any other Rpackage to ensure and maintain sustainability.

The AMR package supports multiple languages. Currently supported languages are English, Dutch, French, German, Italian, Portuguese, and Spanish. The system language will be used if the language is supported but can be overwritten with `options(AMR_locale = ...)`. Multi-language support affects language-dependent output of functions such as `mo_name()`, `mo_gramstain()`, `mo_type()`, and `ab_name()`.

The AMR package uses S3 classes, object oriented (OO) systems available in R. They allow different types of output based on the user input. The AMR package introduces 5 S3 classes ('`mo`', '`ab`', '`rsi`', '`mic`', and '`disk`') to increase the convenience when working with antimicrobial susceptibility data.

## 4.7 Reproducible example

We consider the problem of working with antimicrobial resistance data from three different hospitals between 2011-01-01 and 2020-01-01. After loading the AMR package and additional tidyverse packages to allow transformation and plotting, we load the `example_isolates_unclean` example data from the AMR package into the global environment and assign it a new name.

```
library("dplyr")
library("tidyverse")
library("AMR")

options(AMR_locale = "en")

data <- example_isolates_unclean

glimpse(data)
#> Rows: 3,000
#> Columns: 8
#> $ patient_id <chr> "J3", "R7", "P3", "P10", "B7", "W3", "J8", "M3", ...
#> $ hospital    <chr> "A", "A", "A", "A", "A", "A", "A", "A", "A", ...
#> $ date        <date> 2012-11-21, 2018-04-03, 2014-09-19, 2015-12-10, ...
#> $ bacteria    <chr> "E. coli", "K. pneumoniae", "E. coli", "E. coli", ...
#> $ AMX         <chr> "R", "R", "R", "S", "S", "R", "R", "R", "S", "S", ...
#> $ AMC         <chr> "I", "I", "S", "I", "S", "S", "S", "S", "S", "S", ...
#> $ CIP         <chr> "S", "S", "S", "S", "S", "R", "S", "S", "S", "S", ...
#> $ GEN         <chr> "S", ...

unique(data$hospital)
#> [1] "A" "B" "C"

unique(data$bacteria)
#> [1] "E. coli"                  "K. pneumoniae"
#> [3] "S. aureus"                "S. pneumoniae"
```

```
#> [5] "klepne"                      "strpne"
#> [7] "esccol"                       "staaur"
#> [9] "Escherichia coli"            "Staphylococcus aureus"
#> [11] "Streptococcus pneumoniae"    "Klebsiella pneumoniae"

data %>%
  count(bacteria)
#>          bacteria   n
#> 1           E. coli 494
#> 2           esccol 508
#> 3       Escherichia coli 516
#> 4           K. pneumoniae 108
#> 5      Klebsiella pneumoniae 102
#> 6           klepne 116
#> 7           S. aureus 247
#> 8           S. pneumoniae 151
#> 9           staaur 240
#> 10      Staphylococcus aureus 243
#> 11 Streptococcus pneumoniae 139
#> 12           strpne 136
```

The data contains 3,000 observations of 8 variables from 3 hospitals. The “bacteria” variable comprises 12 unique elements. However, they appear to encode the same information in different formats ('E. coli', 'K. pneumoniae', 'S. aureus', 'S. pneumoniae', 'klepne', 'strpne', 'esccol', 'staaur', 'Escherichia coli', 'Staphylococcus aureus', 'Streptococcus pneumoniae', 'Klebsiella pneumoniae'). We can use the `as.mo()` function to standardise the bacterial codes and add a variable with the official scientific name. The correct transformation of the bacterial codes can be reviewed by calling the `mo_uncertainties()` function.

```
data <- data %>%
  mutate(bacteria = as.mo(bacteria),
         bacteria_name = mo_name(bacteria))

mo_uncertainties()
#> "E. coli" -> Escherichia coli (B_ESCHR_COLI, matching score =
#>                         0.688)
#>           Also matched: Entamoeba coli (0.079)
#> "K. pneumoniae" -> Klebsiella pneumoniae (B_KLBSL_PNMN, matching
#>                         score = 0.786)
#>           Also matched: Klebsiella pneumoniae ozaenae
#>                         (0.707), Klebsiella pneumoniae rhinoscleromatis
#>                         (0.658)
#>
#> "S. aureus" -> Staphylococcus aureus (B_STPHY_AURS, matching score
```

```
#> = 0.690)
#> Also matched: Staphylococcus aureus anaerobius
#> (0.625), Streptomyces aureus (0.355), Stentor aureus
#> (0.052)

data %>%
  count(bacteria, bacteria_name)
#>      bacteria      bacteria_name   n
#> 1 B_ESCHR_COLI    Escherichia coli 1518
#> 2 B_KLBSL_PNMN   Klebsiella pneumoniae 326
#> 3 B_STPHY_AURS   Staphylococcus aureus 730
#> 4 B_STRPT_PNMN   Streptococcus pneumoniae 426
```

In a next step, we can further enrich the data with additional microbial taxonomic data based on the “bacteria” variable, such as Gram-stain and microorganism family.

```
data <- data %>%
  mutate(gram_stain = mo_gramstain(bacteria),
        family = mo_family(bacteria))

data %>%
  count(gram_stain)
#>      gram_stain   n
#> 1 Gram-negative 1844
#> 2 Gram-positive 1156

data %>%
  count(family)
#>      family   n
#> 1 Enterobacteriaceae 1844
#> 2 Staphylococcaceae 730
#> 3 Streptococcaceae 426
```

The variables “AMX”, “AMC”, “CIP”, and “GEN” contain antimicrobial susceptibility test results. The abbreviations stand for the tested antimicrobial agent. The official names and additional information about the antimicrobial agents can be checked with the `ab_info()` function from the `AMR` package.

```
ab_info("AMX")
#> $ab
#> [1] "AMX"
#>
#> $atc
#> [1] "J01CA04"
#>
```

```

#> $cid
#> [1] 33613
#>
#> $name
#> [1] "Amoxicillin"
#>
#> $group
#> [1] "Beta-lactams/penicillins"
#>
#> $atc_group1
#> [1] "Beta-lactam antibacterials, penicillins"
#>
#> $atc_group2
#> [1] "Penicillins with extended spectrum"
#>
#> $tradenames
#> [1] "actimoxi" "amoclen" "amolin"
#> [4] "amopen" "amopenixin" "amoxibiotic"
#> [7] "amoxicaps" "amoxicilina" "amoxicillin"
#> [10] "amoxicilline" "amoxicillinum" "amoxiden"
#> [13] "amoxil" "amoxivet" "amoxy"
#> [16] "amoxyccillin" "anemolin" "aspenil"
#> [19] "biomox" "bristamox" "cemoxin"
#> [22] "clamoxyll" "delacillin" "dispermox"
#> [25] "efpenix" "flemoxin" "hiconcil"
#> [28] "histocillin" "hydroxyampicillin" "ibiamox"
#> [31] "imacillin" "lamoxy" "metafarma capsules"
#> [34] "metifarma capsules" "moxacin" "moxatag"
#> [37] "ospamox" "pamoxicillin" "piramox"
#> [40] "robamox" "sawamox pm" "tolodina"
#> [43] "unicillin" "utimox" "vetramox"
#>
#> $ddd
#> $ddd$oral
#> $ddd$oral$amount
#> [1] 1.5
#>
#> $ddd$oral$units
#> [1] "g"
#>
#>
#> $ddd$iv
#> $ddd$iv$amount
#> [1] 3
#>

```

```
#> $ddd$iv$units
#> [1] "g"
```

In a data set containing antimicrobial names or codes (e.g., antimicrobial prescription data), the `as.ab()` function can be used to transform all values to valid antimicrobial codes. Extra columns with the official name and the defined daily dose (DDD) for intravenous administration could be added using `ab_name()` and `ab_ddd()`.

```
antimicrobial_example <- data.frame(agents = c("AMX",
                                                "Ceftriaxon",
                                                "Cipro"))

antimicrobial_example %>%
  mutate(agents = as.ab(agents),
        agent_names = ab_name(agents),
        ddd_iv = ab_ddd(agents, administration = "iv"))
#> agents agent_names ddd_iv
#> 1 AMX Amoxicillin 3.0
#> 2 CRO Ceftriaxone 2.0
#> 3 CIP Ciprofloxacin 0.8
```

Coming back to the cleaning of the data, the columns for the antimicrobial susceptibility test results (“AMX”, “AMC”, “CIP”, “GEN”) need to be checked to contain only standard values (“R”, “S”, “I”).

```
data %>%
  select(AMX:GEN) %>%
  pivot_longer(everything(),
               names_to = "antimicrobials",
               values_to = "interpretation") %>%
  count(interpretation)
#> # A tibble: 4 x 2
#>   interpretation     n
#>   <chr>           <int>
#> 1 < 0.5 S            143
#> 2 I                 1105
#> 3 R                 4607
#> 4 S                 6145
```

The values contain some mixed values. The `as.rsi()` function can be used to clean these values and to assign a new class ('rsi') for further use of AMR functions.

```
data <- data %>%
  mutate_at(vars(AMX:GEN), as.rsi)
```

```
data %>%
  select(AMX:GEN) %>%
  pivot_longer(everything(),
               names_to = "antimicrobials",
               values_to = "interpretation") %>%
  count(interpretation)
#> # A tibble: 3 x 2
#>   interpretation     n
#>   <rsi>           <int>
#> 1 S                6288
#> 2 I                1105
#> 3 R                4607
```

After this transformation, the `eucast_rules()` function can be applied to apply the latest resistance reporting guidelines.

```
data <- data %>%
  eucast_rules()
```

The output to the console lists the changes made to data:

```
#> The rules affected 508 out of 3,000 rows, making a total of 657 edits
#> => added 0 test results
#>
#> => changed 657 test results
#> - 11 test results changed from "S" to "I"
#> - 473 test results changed from "S" to "R"
#> - 85 test results changed from "I" to "R"
#> - 19 test results changed from "I" to "S"
#> - 33 test results changed from "R" to "I"
#> - 36 test results changed from "R" to "S"
```

The data is now clean and ready for further analysis, for example, the identification of multi-drug resistant microorganisms. In this example, we use the Dutch guideline to determine multi-drug resistance [35].

```
data <- data %>%
  mutate(mdro = mdro(., guideline = "nl"))

data %>%
  count(bacteria_name, mdro)
#>          bacteria_name      mdro     n
#> 1    Escherichia coli Negative 1123
#> 2    Escherichia coli Positive  395
#> 3 Klebsiella pneumoniae Negative  237
#> 4 Klebsiella pneumoniae Positive   89
#> 5 Staphylococcus aureus Negative  730
```

```
#> 6 Streptococcus pneumoniae Negative 426
```

According to the Dutch guideline, 484 (395 + 89) multi-drug resistant microorganisms were found in 3,000 tested isolates. No multi-drug resistance was found in *Staphylococcus aureus* and *Streptococcus pneumoniae*.

As described in Section 4.4.1, the identification of first isolates is essential for the reporting of resistance patterns. Using the `filter_first_isolate()` function and `proportion_df()` in combination with `group_by()`, we get a complete resistance analysis per hospital, bacteria, first isolate, and tested antimicrobial agent in one call:

```
resistance_proportion <- data %>%
  filter_first_isolate() %>%
  group_by(hospital) %>%
  proportion_df()

head(resistance_proportion)
#>   hospital          antibiotic interpretation    value
#> 1      A            Amoxicillin           SI 0.5773050
#> 2      A            Amoxicillin           R 0.4226950
#> 3      A Amoxicillin/clavulanic acid       SI 0.8085106
#> 4      A Amoxicillin/clavulanic acid       R 0.1914894
#> 5      A            Ciprofloxacin          SI 0.8042553
#> 6      A            Ciprofloxacin          R 0.1957447
```

From the console we get the information how many first isolates were identified and used in the filter.

From here on, the data is ready for further analysis with functions for plotting (e.g., the `ggplot2` package [41]), AMR extension functions for base R (e.g., `summary()`, `plot()`), or AMR helper functions for plotting and basic modelling (e.g., `ggplot_rsi()`, `geom_rsi()`, `resistance_predict()`).

## 4.8 Discussion

For the first time, a free and open-source software solution is available to cover all aspects of working with antimicrobial resistance data. The `AMR` package provides functionalities that enable standardised and reproducible workflows from raw laboratory data to publishable results, for research and clinical workflows alike. In the field of clinical microbiology and infectious diseases, research and clinical workflows are closely linked. For example, a performed research study on the prevalence of antimicrobial-resistant bacteria can have direct implications on the choice of antimicrobial agents for the treatment of patients. The `AMR` package was developed to be used in any research or clinical setting where the data analysis on microorganisms, antimicrobial resistance, antimicrobial agents is required.

Both, researchers and clinicians rely on the data from electronic laboratory information systems (LIS) where laboratory test results are processed, stored, and archived. Although some commercial solutions exist to conduct medical microbiological data analysis, these solutions are not comprehensive enough to apply antimicrobial resistance analysis for any clinical or research setting. Costs of these tools are a further constraint in resource-limited settings. Moreover, researchers and clinicians that require data from multiple LIS sources to be used in multi-center studies experience major barriers which cannot be solved by available commercial solutions.

Firstly, simple codes for microorganisms show substantial differences between different LIS and presumably correct taxonomic names are often misspelled or outdated. We analysed the taxonomic names of bacteria used in reports from seven different public health institutions that perform microbiological diagnostics in the Netherlands and compared them with an official scientific up-to-date source for microbial taxonomy, the Catalogue of Life [16]. These institutions cover microbiological diagnostics for hospitals and primary care for 15% of the total Dutch population. All institutions reported outdated taxonomic names with a maximum lag ranging between 34 and 41 years. Given that antimicrobial resistance guidelines are strongly based on the microbial taxonomy (some rules only apply to a specific genus, other rules apply to a specific family), it is crucial that this information is correct and timely updated. All institutions admitted that there was no standard operating procedure to maintain their taxonomic reference data. Implementing and maintaining the taxonomic data for these and other institutions has been challenging, since no common machine-readable, reliable and up-to-date resource for the microbial taxonomy was publicly available. For reliable reference data about antimicrobial agents, this also holds true. The **AMR** package provides machine-readable reference data files for the complete microbial taxonomy and for more than 500 antimicrobial agents. Using functions starting with `mo_*` and `ab_*`, names of microorganisms and antimicrobial agents can be translated between different LIS codes or other forms of text codes for microorganisms and consequently allows to merge data sets from different sites with little effort.

Secondly, antimicrobial resistance interpretation guidelines [5,6] and taxonomic definitions of microorganisms are under constant change and are continually published in dedicated peer-reviewed journals. This is further complicated by differences between local, regional, and national guidelines. Yet, comparability and reproducibility across setting and time are key in research and clinics. The **AMR** package functions `eucast_rules()` (to apply guidelines to data), `mdro()` (to check for multi-drug resistance according to guidelines), or `first_isolate()` (to determine first isolates according to guidelines) address the needs to standardise comparability, and empower data analysts beyond the capabilities of their local LIS. The **AMR** package can be used as an extra layer of data validation when retrieving raw data from a LIS. Overall, the functionality of the **AMR** package has the potential to improve data validity in clinical settings, to ease multi-center study workflows, and to foster research reporting practices. The in-

herent global nature of antimicrobial resistances requires researchers, clinicians, and policy makers to reach beyond the borders of their local laboratory. The **AMR** package can build the bridge to link these sources and further encourages open science principles through its open-source approach.

The **AMR** package also has limitations. It does not introduce novel statistical tests or models, nor does it add additional analytical approaches for AMR research. The calculation of the proportion of susceptibility for more than one antimicrobial agent simultaneously (see Section 4.5.1) seems simple but is subject to unclear reporting in clinical practice [42,43]. The lack of clearly defined algorithms can lead to the effect that co-resistance rates for more than one antimicrobial agent are dropped altogether [44]. The inclusion of isolates that are tested for some agents (only\_all\_tested = FALSE) or only isolates tested for all agents (only\_all\_tested = TRUE) can have an imminent clinical impact on patient care, if one combination of antimicrobial agents is preferred over another. Therefore, the **AMR** package provides different algorithms to standardise this crucial calculation. Unfortunately, unambiguous methodology for determining the right algorithm is lacking in scientific literature. An analysis on the algorithms used in the **AMR** package and their clinical impact is in preparation.

Reliable information about antimicrobial resistance is vital for clinical decision-making in infectious diseases, since the outcome of local antimicrobial resistance analyses support medical professionals/clinicians in the treatment choices for their patients. Moreover, when this information can be reliably stratified by, for example, year, hospital, and type of patients, new information can lead to new insights for choosing the best antimicrobial therapy for patients suffering from infections. The **AMR** package enables this by providing all required analysis tools and can therefore empower decision-making in infectious management. The **AMR** package is already being applied to this end in six hospitals in the Netherlands. The choice of empirical antimicrobial treatment (meaning; choosing the initial therapy at a time of not knowing the infection-causing pathogen) for septic non-post-surgical patients has been altered in at least one Dutch hospital, by analysing antimicrobial resistance data with the **AMR** package. The clinical effect of this adjustment is being studied at the moment. To improve the quality of such analyses, planned future developments comprise the implementations of an imputation algorithm specifically for antimicrobial agents, and method guidance for applying prediction modelling in a health care setting based on patient-specific properties.

Since the first package release, users from different public and private settings have been suggesting additional functionalities, in particular, the incorporation of country- or time- specific guidelines (e.g., Magiorakos *et al.* [31]). This community-centred development will be continued and maintained by researchers at the University Medical Center Groningen and data scientists at Certe Medical Diagnostics and Advice, both non-profit public health organisations located in Groningen, the Netherlands. Moreover, a group of contributors from five different Dutch health care institutions has been formed at the Dutch

Association for Medical Microbiology (Nederlandse Vereniging voor Medische Microbiologie - NVMM) that also peer-review major changes to the package, including the implementation of guideline updates. This way, updates required for scientific developments as well as maintaining consistent reproducibility are ensured. Updates to databases and guidelines included in the **AMR** package are incorporated on a regular and automated basis, while preserving version control. Any function making use of guidelines (e.g., `eucast_rules()`) refers to the latest implemented version of the guideline by default.

The aim of the **AMR** package is to provide a comprehensive toolbox of solutions for antimicrobial resistance data processing and analysis on an institution- and country-independent scale for clinical practice and research that are required according to international standards, but were not available to date.

## Computational Details

The results in this paper were obtained using R4.0.2 in RStudio 1.3.1093 [45] with the **AMR** package 1.5.0, running under macOS Catalina 10.15.

Ritself and all packages used are available from the Comprehensive RArchive Network (CRAN) at <https://CRAN.R-project.org/>. All development versions of the **AMR** package are available at <https://github.com/msberends/AMR/>.

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## Appendix A: Included Data Sets

- **microorganisms**

A ‘`data.frame`’ containing 67,151 (sub)species with 16 columns comprising their complete microbial taxonomy according to the Catalogue of Life [16]. Included microorganisms and their complete taxonomic tree of all included (sub)species from kingdom to subspecies with year of scientific publication and responsible author(s):

- All 55,415 (sub)species from the kingdoms of Archaea, Bacteria, Chromista and Protozoa;
- All 9,582 (sub)species from these orders of the kingdom of Fungi: Eu-rotiales, Onygenales, Pneumocystales, Saccharomycetales, Schizosaccharomycetales and Tremellales;
- All 2,153 (sub)species from 47 other relevant genera from the kingdom of Animalia (like *Strongyloides* and *Taenia*);
- All 12,708 previously accepted names of included (sub)species that have been taxonomically renamed.

The kingdom of Fungi is a very large taxon with almost 300,000 different (sub)species, of which most are not microbial (but rather macroscopic, such as mushrooms). Therefore, not all fungi fit the scope of the `AMR` package. By only including the aforementioned taxonomic orders, the most relevant fungi are covered (such as all species of *Aspergillus*, *Candida*, *Cryptococcus*, *Histoplasma*, *Pneumocystis*, *Saccharomyces* and *Trichophyton*).

- **antibiotics**

A ‘`data.frame`’ containing 456 antibiotic agents with 14 columns. All entries in this data set have three different identifiers: a human readable EARS-Net code (as used by ECDC [19] and WHONET [46] and primarily used by this package), an ATC code (as used by the WHO [21]), and a CID code (Compound ID, as used by PubChem [23]). The data set contains more than 5,000 official brand names from many different countries, as found in PubChem. Other properties in this data set are derived from one or more of these codes, such as official names of pharmacological and chemical subgroups, and defined daily doses (DDD).

- **antivirals**

A ‘`data.frame`’ containing 102 antiviral agents with 9 columns. Like the `antibiotics` data set, it contains ATC codes (as used by the WHO [21]), and a CID code (Compound ID, as used by PubChem [23]), as well as the

official name and defined daily dose (DDD) for each antiviral agent.

- **example\_isolates**

A ‘`data.frame`’ containing test results of 2,000 microbial isolates. The data set reflects real patient data and can be used to practice AMR analysis. It is structured in the typical format of laboratory information systems with one row per isolate and one column per tested antimicrobial agent (i.e., an antibiogram).

- **example\_isolates\_unclean**

A ‘`data.frame`’ containing test results of 3,000 microbial isolates that require cleaning up before they can be used for analysis. This data set can be used to practice AMR analysis and is featured in section 4.7.

- **WHONET**

A ‘‘`data.frame`’ containing 500 observations and 53 columns, with the exact same structure as an export file from WHONET 2019 software [46]. Such files can be used with the `AMR` package, as this example data set demonstrates. The antibiotic test results are from the `example_isolates` data set. All patient names are created using online surname generators and are only in place for practice purposes.



## Chapter 5

# Rapid Analysis of Diagnostic and Antimicrobial Patterns in R (RadaR): Interactive Open-Source Software App for Infection Management and Antimicrobial Stewardship

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## Abstract

Analysing process and outcome measures for all patients diagnosed with an infection in a hospital, including those suspected of having an infection, requires not only processing of large datasets but also accounting for numerous patient parameters and guidelines. Substantial technical expertise is required to conduct such rapid, reproducible, and adaptable analyses; however, such analyses can yield valuable insights for infection management and antimicrobial stewardship (AMS) teams. The aim of this study was to present the design, development, and testing of RadaR (Rapid analysis of diagnostic and antimicrobial patterns in R), a software app for infection management, and to ascertain whether RadaR can facilitate user-friendly, intuitive, and interactive analyses of large datasets in the absence of prior in-depth software or programming knowledge. RadaR was built in the open-source programming language R, using Shiny, an additional package to implement web-app frameworks in R. It was developed in the context of a 1339-bed academic tertiary referral hospital to handle data of more than 180,000 admissions. RadaR enabled visualisation of analytical graphs and statistical summaries in a rapid and interactive manner. It allowed users to filter patient groups by 17 different criteria and investigate antimicrobial use, microbiological diagnostic use and results including antimicrobial resistance, and outcome in length of stay. Furthermore, with RadaR, results can be stratified and grouped to compare defined patient groups on the basis of individual patient features. AMS teams can use RadaR to identify areas within their institutions that might benefit from increased support and targeted interventions. It can be used for the assessment of diagnostic and therapeutic procedures and for visualizing and communicating analyses. RadaR demonstrated the feasibility of developing software tools for use in infection management and for AMS teams in an open-source approach, thus making it free to use and adaptable to different settings.

## 5.1 Introduction

### 5.1.1 Background

With antimicrobial resistance (AMR) on the rise, efforts are being made worldwide to focus on the preservation of antimicrobials as a precious non-renewable resource. Infection management in the form of antimicrobial stewardship (AMS) programs has emerged as an effective solution to address this global health problem in hospitals. AMS programs are defined as “a coherent set of actions which promote using antimicrobials responsibly” [1]. Stewardship interventions and activities focus on individual patients (personalised medicine and consulting) as well as patient groups or clinical syndromes (guidelines, protocols, information technology infrastructure, and clinical decision support systems) while prioritizing improvement in quality of care and patient safety for any intervention. The appropriate use of antimicrobials based on accurate and timely diagno-

tics is integral for the successful management of infections. In doing so, the diagnostics contribute to efforts in minimizing AMR by optimizing the use of antimicrobials.

AMS setups in hospitals are often heterogeneous, but audit and feedback to assess the goals are essential parts of most programs, and they are included in international guidelines and reviews [2-7]. Important data for AMS programs include, for example, days of therapy (DOT), daily defined doses (DDD), admission dates, length of stay (LOS), and adherence to local or national diagnostic, therapeutic, or infection management guidelines [1]. Clinical outcomes, quality of care, or consumption of hospital resources can be measured, for example, using mortality data or surrogate parameters such as LOS. The collection of these data is facilitated by electronic health records (EHRs) and administrative local databases. Notably, administrative data have also been shown to be a reliable source for assessing clinical outcomes [8].

EHRs usually offer quick insights into useful infection management data on the individual patient level. However, easy access to analyse patient groups (e.g., stratified by departments or wards, specific antimicrobials, or diagnostic procedures used) is difficult to implement in daily practice. It is even more challenging to rapidly analyse larger patient populations (e.g., spread over multiple specialties) even though this information might be available. Nevertheless, this is vital for meaningful analysis, including possible confounders and pattern recognition across different populations. Moreover, when aggregated data are available, it is often not possible to trace individual patients, and analyses lack the ability to be further adjusted or stratified.

AMS teams are multidisciplinary, and they act beyond the borders of single specialties [9]. They are usually understaffed, with limited data analysis support [10,11]. Therefore, they need user-friendly and time-saving data analysis resources, without the need for profound technical expertise once the system is set up. Aggregating and linking data of antimicrobial use, guideline adherence, and clinical outcomes at the institutional level can build the basis for important insights for these teams. These could be used to identify areas within hospitals that might benefit most from supportive AMS interventions (e.g., subspecialties with lower guideline adherence or unusual patterns of antimicrobial use). Moreover, feedback from these data could help physicians better understand their patient population as a whole; in addition, hospital administration could allocate resources in a more targeted fashion.

Furthermore, aggregated data and simultaneous analysis of multiple areas (e.g., use of diagnostics and antimicrobials) present an extensive insight into large patient populations. This also enables the development of comprehensive and multidisciplinary approaches of infection management, combining diagnostic and therapeutic perspectives [1,9,12]. Unfortunately, these kinds of analyses still require substantial statistical knowledge and software skills, and it is time consuming when performed.

Technology, data science, and software app development can bring solutions to complex data handling problems such as those described above. Software app development for medical and epidemiological (research) questions has found many important answers during recent years. For example, software apps at hospital emergency departments (EDs) in the form of a dashboard have been shown to improve efficiency and quality of care for patients requiring emergency admission to hospital [13]. These software apps are used to communicate clearly defined clinical problems, such as mortality ratio, number of cardiac arrests, or readmission rate to the EDs. This has led to a decreased LOS and mortality at the EDs. Others used similar approaches to rapidly and interactively display geographical locations of tuberculosis cases without the need of technical expertise improving the understanding of transmission and detection [14]. Furthermore, data-driven fields such as genomics are front runners in developing new, innovative software apps to handle large datasets, in close collaboration with bioinformatics [15].

It is important to note that all of these abovementioned software apps have been created in an open-source approach. This means that the underlying source code can be easily shared, easily modified, and freely distributed through open repositories, such as GitHub [16], taking open-source software license obligations into account. This facilitates collaboration, quality control through code review, and easy adaptation to many different settings and information technology systems, and this supports the use of advanced data visualisation for users with minimal experience in programming and little or no budget for professional database engineers [15].

In the field of medical microbiology, different approaches have been described to interactively work with microbiological diagnostics data and EHRs: electronic antibiograms, centralised resistance analysis, EHR data mining, and clinical decision support systems for AMS are great examples for innovation in the field [17-19]. However, a full open-source approach for software apps working with combined antimicrobials use and diagnostic data of individual patients on the hospital level in the field of infection management is still lacking.

### 5.1.2 Objectives

We followed principles of open knowledge [20] to address the need for an interactive, easy-to-use software app that allows users to investigate antimicrobial use, microbiological diagnostic use, and patient outcomes at an institutional (hospital) level. We developed an open-source, web-based software app – Rapid analysis of diagnostic and antimicrobial patterns in R (RadaR) that can be used for AMS and infection management. This free software app can be run on regular computers or implemented on local or web-based servers to be accessed through standard web browsers. The focus user group of this software app is health care professionals involved in AMS (e.g., infectious disease specialists, clinical microbiologists, and pharmacists). Although some technical expertise (basic R knowledge) is needed for installation and implementation, the use of

RadaR follows usual web browser user experiences. RadaR enables rapid and reproducible data analysis without extensive previous analysis expertise in a graphically appealing way while being adaptable to different settings. RadaR's analyses are based on datasets of individual patients. Therefore, aggregated results can also be stripped down, and additional patient features can be investigated. With this software app, we aim at supporting data-driven hospital insights and decision making for actors in the field of AMS in a free, transparent, and reproducible way.

## 5.2 Methods

For the development of software in an open-source environment, we used the open-source programming language R in conjunction with RStudio version 1.1.463 (RStudio, Inc) [21], an open-source integrated desktop environment for R [22]. Both R and RStudio are free of charge, and they need to be installed for the development and implementation of RadaR. To build RadaR as a web-based software app, we used the Shiny package for R [23]. Shiny allows R users to build interactive web apps without extensive knowledge in web design and its programming languages. The web apps can be run and hosted on the web for free [24], as well as on local or cloud-based servers or on personal computers.

The functionality of R can be easily extended by installing additional packages. All packages used for the development of RadaR are listed in Table 1. RadaR is developed in an open-source environment and licensed under GNU General Public License v2.0 [25], giving options to change, modify, and adapt RadaR to both personal and commercial users' needs while requiring the need to document code changes [25].

RadaR's calculations and data aggregation are done reactively on the basis of the selection of the user. Single observations on the patient level build the basis for any calculation. RadaR uses common CSV files as input. A total of three different data sources are read in RadaR for admission, antimicrobial, and microbiological data, which are merged and transformed upon start. A patient number or study number is used as a unique identifier. All antimicrobial and microbiological data are checked to ascertain whether they fall in the interval of admission dates.

Table 1. Required R packages for RadaR.

The input data should be structured in a dataset format, where each variable is one column and each observation is one row. This follows the concept of "tidy data," as defined by Hadley Wickham [26]. Table 2 displays the set of variables underlying RadaR's functionality. In our setting for the development of RadaR, these variables originated from three different data sources: administrative data from the hospital data warehouse, microbiological data from the laboratory information system, and antimicrobial prescription data from the computerised

R package	Minimal version
AMR	0.5.0
data.table	1.11.6
DT	0.4.0
ggridges	0.5.0
lubridate	1.7.4
plotly	4.8.0
qicharts2	0.5.1
rintrojs	0.2.0
shiny	1.1.0
shinyBS	0.61
shinycssloaders	0.2.0
shinydashboard	0.7.0
shinyjs	1.0.0
shinyWidgets	0.4.3
survival	2.42-6
survminer	0.4.3
tidyverse	1.2.1
viridis	0.5.1
zoo	1.8-3

prescriber order entry system. The data preparation and cleaning process are very specific for each data source, dependent on local data standards, and difficult to generalise. Therefore, Table 2 represents the final variables and formats for the analysis and use with RadaR, referring to the “tidy data” concept above and to the R package collection tidyverse for the preparation process [26,27]. Additional variables are calculated and transformed using the packages lubridate and zoo for time points and intervals, and AMR for antimicrobial (group) names, microbial isolate names, first isolate identification, and resistance analysis [28-30]. Microbiological resistance is calculated per antimicrobial substance or as co-resistance if more than one substance is selected.

Table 2. Input variables for RadaR.

The input data should be structured in a dataset format, where each variable is one column and each observation is one row. This follows the concept of “tidy data,” as defined by Hadley Wickham [26]. Table 2 displays the set of variables underlying RadaR’s functionality. In our setting for the development of RadaR, these variables originated from three different data sources: administrative data from the hospital data warehouse, microbiological data from the laboratory information system, and antimicrobial prescription data from the computerised prescriber order entry system. The data preparation and cleaning process are very specific for each data source, dependent on local data standards, and difficult to generalise. Therefore, Table 2 represents the final variables and formats for the analysis and use with RadaR, referring to the “tidy data” concept above

Variable	Detail
<b>Admission data</b>	
<code>adm_end_date</code>	Discharge date (a)
<code>adm_id</code>	Admission ID
<code>adm_route</code>	Origin
<code>adm_start_date</code>	Admission date (a)
<code>birth_date</code>	Birth date (a)
<code>death_during_adm</code>	In-hospital death (TRUE/FALSE)
<code>gender</code>	Gender
<code>id</code>	Patient ID or study ID
<code>specialty</code>	General specialty (internal medicine, surgery, and other)
<code>sub_specialty</code>	Subspecialty
<b>Antimicrobial data</b>	
<code>ab_route</code>	Administration route
<code>ab_start_date</code>	Start of antimicrobial (a)
<code>ab_stop_date</code>	Stop of antimicrobial (a)
<code>atc_code</code>	Fifth level of the World Health Organization Anatomical Therapeutic Chemical (WHO ATC) classification system (b)
<code>ddd_per_day</code>	Defined daily dose of antimicrobial according to WHO ATC classification system per day (b)
<code>id</code>	Patient ID or study ID
<b>Microbiological data</b>	
Antimicrobial susceptibility testing	Several columns of tested antimicrobial agents (e.g., amoxicillin, ciprofloxacin) with resistance results (R/I/S)
<code>id</code>	Patient ID or study ID
<code>material</code>	Test material
<code>mo</code>	Microbial ID (if test = positive) (c)
<code>specialty</code>	Ordering specialty
<code>test_date</code>	Test date (a)

a) YYYY-MM-DD

b) As available on the website [31]

c) As defined by the `AMR` package for `R` [30]

and to the R package collection tidyverse for the preparation process [26,27]. Additional variables are calculated and transformed using the packages lubridate and zoo for time points and intervals, and AMR for antimicrobial (group) names, microbial isolate names, first isolate identification, and resistance analysis [28-30]. Microbiological resistance is calculated per antimicrobial substance or as co-resistance if more than one substance is selected.

RadaR can be used for graphical exploratory data analysis. Differences in LOS are displayed by a Kaplan-Meier curve in conjunction with a log-rank test, using the survminer package [32]. Time trends for number of admissions, antimicrobial consumption, and resistance counts per year, quarter, or month, are visualised in run charts using the qicharts2 package [33]. Nonrandom variation in these run charts is tested using Anhøj's rules [34].

RadaR has been developed in macOS High Sierra (1.4 GHz, 4 GB RAM), and it was successfully tested in Windows 7 (3.2 GHz, 8 GB RAM) and Linux (Ubuntu 16.04.4 LTS, 3.4 GHz, 12 GB RAM). A running example version has been deployed to shinyapps.io, a publicly available web hosting service for R Shiny apps [35]. The entire source code of RadaR is freely accessible on GitHub [36]. We intend to integrate suggestions and feedback coming from its users and the R community. RadaR was developed using data of patients admitted to the University Medical Center Groningen, Groningen, the Netherlands. Data were collected retrospectively, and permission was granted by the ethical committee (METc 2014/530). RadaR can be used locally in protected environments or hosted on the web, provided appropriate measures have been taken to guarantee data protection, depending on national regulations.

## 5.3 Results

### 5.3.1 Overview

We have developed RadaR, a web-based software app providing an intuitive platform for rapid analysis of large datasets containing information about patients' admission, antimicrobial use, and results of microbiological diagnostic tests. This software app can help users (i.e., AMS team members) find answers to questions, such as "What are the most commonly used antimicrobials at an institution/specialty/department and have they changed over time?", "Were adequate microbiological diagnostics performed at the start of antimicrobial treatments?", "What are the most frequent microorganisms found and their resistance patterns in different departments?", and "Can we identify priority areas within a hospital where antimicrobial or microbiological diagnostic use has the largest room for improvement?".

### 5.3.2 Application Design

RadaR is designed in the form of a web browser-based dashboard that most users are familiar with from typical websites and web-based tools (see Figure 1). The basis of RadaR's functionality is filtering datasets and producing analytical graphs according to selection criteria defined by the user. Any calculations and data aggregation are based on single observations of individual patients. To identify and analyse groups of patients, 17 different selection criteria can be found in the sidebar (Table 3). The output of RadaR is grouped into four panels (patient, antimicrobials, diagnostics, and outcome) that each comprise three to four output boxes displaying the results.

Table 3. Selection criteria in sidebar.

Tab name and criteria	Functionality
<b>Antimicrobials</b>	
Start of antimicrobials (in relation to start of admission)	Select patients starting treatment in a defined time period
Minimum duration of treatment (days) all antimicrobials	Select patients with a minimum treatment duration
Minimum duration of prescription (days) single antimicrobial	Define the minimum duration of a prescription for any selected antimicrobial
Administration route	Intravenous or oral
First antimicrobial only	Filter patients for first prescribed antimicrobial only or any (on the basis of all other selection criteria)
Groups of antimicrobials	Fourth level of the WHO ATC classification system (a)
Antimicrobials	Fifth level of the WHO ATC classification system (a)
<b>Patients</b>	
Gender	Female or male
Age	As available in the data
<b>Year</b>	
Year	Years available in the data
<b>Specialty</b>	
Specialty	Internal medicine, surgery, or other
Minimum number of patients per subspecialty	0, 10, 100, 1000 or 10000
Include only this subspecialty	All other subspecialties will be excluded
Exclude subspecialty	Define single subspecialties to be excluded
<b>Origin</b>	
Origin at admission	As available in the data
<b>Diagnostics</b>	
Type of diagnostics	Blood culture or urine culture test
Days to first test (in relation to start of antimicrobials)	Define time period for tests to be performed in

a) As available on the website [31]

All output is based on the selection criteria defined by the user in the sidebar. Each new selection and any change need to be confirmed by clicking the confirm

selection button (see Figure 1). Users can navigate among the different analysis panels by clicking the respective button.

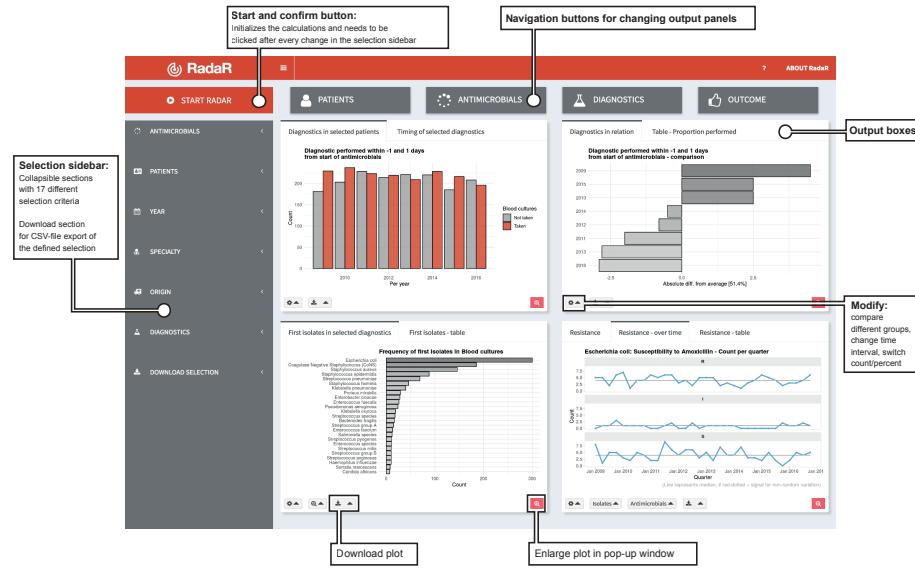


Figure 5.1: Application design.

Results are shown in bar charts, density plots, run charts, a bubble plot, and a Kaplan-Meier curve for LOS in hospital. Each panel further displays a table summarizing the respective data analyses. All output boxes and their content are described in Table 4. Most output boxes include modification options that can be identified by small gear icons (see Figure 1). These clickable icons allow for further specification of the generated plots and tables. Users can compare different groups (e.g., antimicrobial use by antimicrobial agent, resistance patterns per isolate, or LOS by specialty) or modify the plots (e.g., switch from count to proportion, change the chart type, or show or hide the legend). Plots and tables can be downloaded through download buttons as PNG files for plots and CSV, Excel, or PDF files for tables.

Table 4. Output boxes for analysis results.

Finally, two datasets (antimicrobial/admission data and microbiological data) of the user-defined selection can be downloaded from the sidebar menu in a CSV-file format for further analysis (e.g., retrieving a list of patient numbers of the selected patient group).

### 5.3.3 Development Process

RadaR has been developed in close contact with the AMS team and senior consulting specialists at the University Medical Center Groningen, Groningen,

Output panel and output box	Output type	Content	Modification options
<b>Patients</b>			
Subspecialties in selection	Bubble chart (a)	Patients per subspecialty	Show top 10 by number of patients
Subspecialties-table	Table	Total number of patients and per subspecialty	(b)
Patient age	Density plot (distribution)	Age distribution in selection	Group by gender
Number of admissions	Run chart	Count of admissions per time period	Per year, per quarter, or per month
<b>Antimicrobials</b>			
Antimicrobials	Bar chart	(Group of) antimicrobials sorted by prescription, DDD (c), or DOT (d)	Single antimicrobials or groups; Select prescription count, DDD, or DOT per 100 bed days
DDD	Run chart	DDD per 100 bed days per group and per time period	Group by none, specialty, subspecialty, and origin; Per year, per quarter, and per month
DOT	Run chart	DOT per 100 bed days per group and per time period	Group by none, specialty, subspecialty, and origin; Per year, per quarter, and per month
DDD/DOT table	Table	Summary of DDD/DOT per 100 bed days per group	DDD or DOT per 100 bed days; Group by antimicrobial (group), year, specialty, subspecialty, and origin
<b>Diagnostics</b>			
Diagnostics in selected patients	Bar chart	Diagnostics taken versus not taken in specified timespan	Count or proportion; Per year, quarter, or month
Timing of selected diagnostics	Bar chart	Time of diagnostics performed in days after start of treatment	(b)
Diagnostics in relation	Bar chart	Absolute difference from average proportion of selected diagnostics performed	Group by antimicrobial (group), year, specialty, subspecialty, and origin
Table - Proportion performed	Table	Summary of proportion of diagnostics performed	Group by antimicrobial (group), year, specialty, subspecialty, and origin
First isolates in selected diagnostics	Bar chart	First isolates of microorganisms sorted by frequency	Group by antimicrobial (group), year, specialty, subspecialty, and origin; Zoom to select more or less isolates shown in graph
First isolates - table	Table	Frequency table of first isolates	Group by year, specialty, subspecialty, and origin
Resistance analysis	Bar chart	Count or proportion of resistance or co-resistance to selected antimicrobials in selected isolates in "R," "S," and "I" categories	Select isolates; Select antimicrobials; Group by year, specialty, subspecialty, and origin; Select count or proportion
Resistance - over time	Run chart	Count of resistance or co-resistance to selected antimicrobials in selected isolates in "R," "S," and "I" categories over time	Select isolates; Select antimicrobials; Per year, per quarter, or per month
Table	Table	Count or proportion of resistance or co-resistance to selected antimicrobials in selected isolates in "R," "S," and "I" categories	Group by year, month, quarter, specialty, subspecialty, and origin; Select isolates; Select antimicrobials; Select count or proportion
<b>Outcome</b>			
Length of stay	Density plot or histogram	Distribution of length of stay per group	Group by all, gender, year, antimicrobial (group), diagnostics performed, specialty, subspecialty, and origin; Show histogram; Show legend; Spread out to remove overlaps
Length of stay - Kaplan-Meier	Kaplan-Meier curve	Kaplan-Meier curve per group	(Groups shown as selected in Length-of-stay box)
Length of stay - table	Table	Summary of length of stay per group	Group by gender, year, antimicrobial (group), diagnostics performed, specialty, subspecialty, and origin

a) Interactive plot showing additional information when hovering over plot.

b) Not applicable

c) DDD: defined daily doses.

d) DOT: days of therapy

the Netherlands, to meet the needs and requirements of this user group. Subsequently, all members of the European Society of Clinical Microbiology and Infectious Diseases Study Group for Antimicrobial Stewardship (ESGAP) were asked to evaluate and test the software app through a running web-based example of RadaR and by filling out a web-based survey. The ESGAP comprises around 200 members from more than 30 countries worldwide. A total of 12 members from 9 different countries took part in the evaluation. This yielded important information on user experiences with the software app, which in turn led to further improvements that are reflected in the version we presented in this report. In a next phase, RadaR will be tested in different settings of ESGAP members and other interested partners using locally available data (e.g., an 837-bed tertiary care hospital in the Netherlands and a 750-bed tertiary care hospital in Greece).

### 5.3.4 Workflow

RadaR was developed and tested with a dataset of all patients admitted to our institution, a 1339-bed academic tertiary referral hospital, within the years of 2009 to 2016, comprising over 180,000 admissions. For simulation purposes and web-based user testing, we have created a test dataset of 60,000 simulated patients. This sample dataset allows testing of RadaR's functionality, but it does not produce meaningful results.

A typical example workflow with RadaR comprises 6 steps (with examples from the test dataset). They are listed below:

1. Define the selection: For example, patients receiving intravenous second- or third-generation cephalosporins as first treatment for at least two days, starting within the first two days of hospital admission from any specialty in all years in the dataset.
2. Patients' panel: Identify the total number of patients and the subspecialties with the highest number of included patients (e.g., 537 patients selected in total, with 97 patients from internal medicine). Investigate patients' gender and age distribution.
3. Antimicrobials panel: Identify the total use of the initial cefuroxime treatment in DDD and DOT per 100 bed days (e.g., 4.51 and 1.5, respectively). Stratify the results by subspecialty and identify the highest number of DDD and DOT per 100 bed days (e.g., highest use by DDD and DOT in internal medicine).
4. Diagnostics panel: Check if the selected microbiological diagnostic test (e.g., blood culture test) has been performed on the same day as the start of the treatment (defined in the sidebar). Investigate the proportion of tests performed over the years and investigate which subspecialty performs best compared with others (e.g., paediatrics). Check which microorganisms (as first isolates) were found in the selected diagnostic specimens (the most common isolate: *Escherichia coli*). Investigate the proportion of isolates resistant to cefuroxime (8.9%) and analyse the trend over time.

5. Outcome panel: Check for patterns of differences in LOS in the defined patient group by subspecialties or performed diagnostics (e.g., highest mean LOS of 7.8 days in Surgery).
6. Refine the selection: Investigate a subgroup of the original selection. For example, select only the top three subspecialties by number of patients and repeat step 2 to 5.

### 5.3.5 Customisation

For setting up RadaR in a new environment after data preparation, users only need to perform the following four steps:

1. Downloading R and RStudio [21,22], which are free to use and open-source software
2. Download or copy and paste RadaR's source code [36] into three files in RStudio – `global.R`, `server.R`, and `ui.R`
3. In `global.R`, manually edit the paths for the prepared datasets to be imported into RadaR
4. Run the app in RStudio with the calling the function `runApp()` in the console or by clicking the green run app button. This will download and install the required R packages needed for the app if they have not been installed previously, and this will create the final dataset for analysis. The RadaR interface will open in the RStudio viewer pane or in a new window of the standard browser of the user's operating system.

RadaR's appearance has been customised using a cascading style sheets (CSS) script [37] that is loaded into the app upon its start. This script needs to be saved into a subdirectory of the directory of the three main files (`global.R`, `server.R`, and `ui.R`) called `www`. We recommend RStudio's project function to create a single project for RadaR and to store all information in this project directory. Users with experience in using CSS can fully alter RadaR's design by changing the underlying CSS script.

## 5.4 Discussion

### 5.4.1 Principal Findings

We have developed a web-based software app for rapid analysis of diagnostic and antimicrobial patterns that can support AMS teams to tailor their interventions. It has been designed to enhance communication of relevant findings while being easy to use. This also applies to users without extensive prior software skills, as it follows usual web browser user experiences. Moreover, it has been developed using open-source software. It is therefore free to use and accessible for download. In our experience, this system can be adapted to new settings within one day, when the required data (Table 2) are available.

Commercial software for infection management is available (e.g., Epic Antimi-

icrobial Stewardship Module, TREAT Steward). These offer extensive options for filtering, analysing, and visualizing EHRs with real-time connections to hospital data infrastructures and have been shown to be useful in clinical practice [38]. However, it is difficult to compare functionalities of these tools because of their non-open-source nature. This fact, along with the required budget to purchase the software, drastically limits their use. We are convinced that transparent software development can support the adoption of data-driven developments while enhancing optimal quality of care and patient safety, which is crucial in the light of new data-driven developments of using EHRs [39,40].

The global nature of infections further calls to develop software tools applicable in resource-limited settings [41]. Open-source approaches for data analysis, such as RadaR, have advantages over traditional methods, such as Excel or SPSS. Hughes et al described those in their report of a software app for RNA-sequencing data analysis [15]. They highlight aspects that were also fundamental for the development of RadaR. First, R allows transparent, reproducible, and sustainable data analysis through scripts that can easily be shared and changed. This can build the basis for collaboration, and this enforces the spirit of open science (also through the strong collaborative R community on the web). Second, R is open source and free to use; therefore, it also enables use in resource-limited settings. Finally, Shiny empowers users to interact with the data, making even very large datasets quickly interpretable.

Innovative approaches used in supporting infection management by leveraging EHRs are being investigated [17-19]. Reporting on AMR, antimicrobial use, and hospital infections (e.g., for quality assurance) is well established, but it is important to integrate these data sources in an approach that allows detailed filtering options on all input. Merely looking at antimicrobial use alone or comparing aggregated results (e.g., total amount of a specific antimicrobial substance per hospital correlated with the total count of a resistant isolate) will result in loss of information or even misleading interpretation. Detailed data and calculations on the basis of each individual patient are crucial to draw informed conclusions. Unfortunately, the abovementioned infection management approaches [17-19] either depend on additional commercial software for data visualisation or the source code is not openly available. We want to encourage others to turn toward available open-source software solutions, such as R, for an increased potential of collaboration and transparency. However, their strength is the connection to real-time data flows. This enables the prospective use and increases their usability for daily clinical practice. RadaR is currently still limited to retrospective data analysis because of a changing hospital data infrastructure in our setting. Technically, it is feasible to connect R-based software apps such as RadaR to real-time hospital data infrastructures running with clinical data standards [42]. For a start, access to static data extraction is often easier and faster to achieve. RadaR can be used to advocate the use of data visualisation tools and improved accessibility of hospital data sources. Until connection to real-time hospital data is established, RadaR can support users as a stand-alone option for retrospective data analysis in infection management. Next steps will

involve testing in multiple settings and forming a user and research group to continue and expand the use of open-source technology and open science principles in infection management.

#### 5.4.2 Conclusions

RadaR demonstrates the feasibility of developing software tools for infection management and AMS teams in an open-source approach, making it free to use, share, or modify according to various needs in different settings. RadaR has the potential to be a highly useful tool for infection management and AMS in daily practice.

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### Conflicts of interests

None declared.

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## Chapter 6

# Better Antimicrobial Resistance Data Analysis and Reporting in Less Time

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## Abstract

Insights and knowledge about local antimicrobial resistance (AMR) levels and epidemiology are essential to guide optimal decision-making processes in antimicrobial use. However, dedicated tools for reliable and reproducible AMR data analysis and reporting are often lacking. In this study, we aimed at comparing the effectiveness and efficiency of traditional analysis and reporting versus a new approach for reliable and reproducible AMR data analysis in a clinical setting. Ten professionals that routinely work with AMR data were recruited and provided with one year's blood culture test results from a tertiary care

hospital results including antimicrobial susceptibility test results. Participants were asked to perform a detailed AMR data analysis in a two-round process: first using their analysis software of choice and next using previously developed open-source software tools. Accuracy of the results and time spent were compared between both rounds. Finally, participants rated the usability of the tools using the systems usability scale (SUS). The mean time spent on creating a comprehensive AMR report reduced from 93.7 (SD  $\pm 21.6$ ) minutes to 22.4 (SD  $\pm 13.7$ ) minutes ( $p < 0.001$ ). Average task completion per round changed from 56% (SD:  $\pm 23\%$ ) to 96% (SD:  $\pm 5.5\%$ ) ( $p < 0.05$ ). The proportion of correct answers in the available results increased from 37.9% in the first to 97.9% in the second round ( $p < 0.001$ ). The usability of the new tools was rated with a median of 83.8 (out of 100) on the SUS. This study demonstrated the significant improvement in efficiency and accuracy in standard AMR data analysis and reporting workflows through open-source software tools in a clinical setting. Integrating these tools in clinical settings can democratise the access to fast and reliable insights about local microbial epidemiology and associated AMR levels. Thereby, our approach can support evidence-based decision-making processes in the use of antimicrobials.

## 6.1 Introduction

Antimicrobial resistance (AMR) is a global challenge in healthcare, livestock and agriculture, and the environment alike. The silent tsunami of AMR is already impacting our lives and the wave is constantly growing [1,2]. One crucial action point in the fight against AMR is the appropriate use of antimicrobials. The choice and use of antimicrobials has to be integrated into a well-informed decision making process and supported by antimicrobial and diagnostic stewardship programmes [3,4]. Next to essential local, national, and international guidelines on appropriate antimicrobial use, the information on AMR rates and antimicrobial use through reliable data analysis and reporting is vital. While data on national and international levels are typically easy to access through official reports, local data insights are often lacking, difficult to establish, and its generation requires highly trained professionals. Unfortunately, working with local AMR data is often furthermore complicated by very heterogeneous data structures and information systems within and between different settings [5,6]. Yet, decision makers in the clinical context need to be able to access these important data in an easy and rapid manner. Without a dedicated team of epidemiologically trained professionals, providing these insights could be challenging and error-prone. Incorrect data or data analyses could even lead to biased/erroneous empirical antimicrobial treatment policies.

To overcome these hurdles, we previously developed new approaches to AMR data analysis and reporting to empower any expert on any level working with or relying on AMR data [7,8]. We aimed at reliable, reproducible, and transparent AMR data analysis. The underlying concepts are based on open-source software,

making them free to use and adaptable to any setting-specific needs. To specify, we developed a software package for the statistical language R to simplify and standardise AMR data analysis based on international guidelines [7]. In addition, we demonstrated the application of this software package to create interactive analysis tools for rapid and user-friendly AMR data analysis and reporting [8].

However, while the use of our approach in research has been demonstrated [9–12], the impact on workflows for AMR data analysis and reporting in clinical settings is pending. AMR data analysis and reporting are typically performed at clinical microbiology departments in hospitals, in microbiological laboratories, or as part of multidisciplinary antimicrobial stewardship activities. AMR data analysis and reporting require highly skilled professionals. In addition, thorough and in-depth analyses can be time consuming and sufficient resources need to be allocated for consistent and repeated reporting. This is further complicated by the lack of available software tools that fulfil all requirements such as incorporation of (inter-) national guidelines or reliable reference data.

In this study, we aimed at demonstrating and studying the usability of our developed approach and its impact on clinicians' workflows in an institutional healthcare setting. The approach should enable better AMR data analysis and reporting in less time.

## 6.2 Methods

The study was initiated at the University Medical Center Groningen (UMCG), a 1339-bed tertiary care hospital in the Northern Netherlands and performed across the UMCG and Certe (a regional laboratory) in the Northern Netherlands. It was designed as a comparison study to evaluate the efficiency, effectiveness, and usability of a new AMR data analysis and reporting approach [7,8] against traditional reporting.

### 6.2.1 Study setup

The setup of the study is visualised in Figure 1 and is explained in the following sections.

The study was based on a task document listing general AMR data analysis and reporting tasks (Table 1). This list served as the basis to compare effectiveness (solvability of each task for every user) and efficiency (time spent solving each task) of both approaches. Tasks were grouped into five related groups and analyses were performed per group (further referred to as five tasks). A maximum amount of time per task (group) was defined for each task. The list of tasks including correct results is available in Appendix A1.

Table 1. AMR data analysis and reporting tasks.

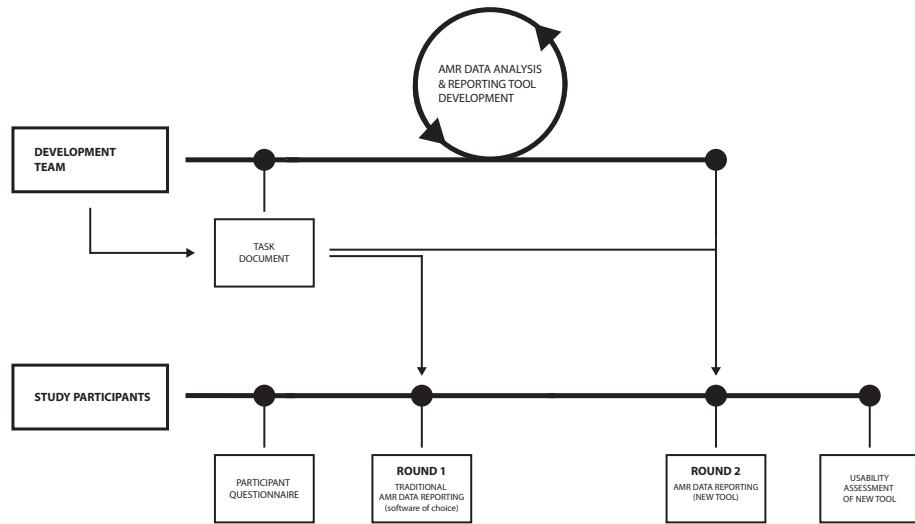


Figure 6.1: Study setup; the same AMR data was used along all steps and rounds.

Task	Task description	Maximum time (minutes)
1	Total number of blood culture sets per year	15
2a	Total number of positive blood culture sets per year	20
2b	Total number of negative blood culture sets per year	
3	Top ten isolated microorganisms per year including isolate count (first isolates*)	20
4a	Resistance profile (S/I & R) in <i>Escherichia coli</i> (first isolates*) for selected antimicrobials	
4b	Resistance profile (S/I & R) in <i>Klebsiella pneumoniae</i> (first isolates*) for selected antimicrobials	30
4c	Resistance profile (S/I & R) in <i>Staphylococcus aureus</i> (first isolates*) for selected antimicrobials	
5a	Empiric success rate for <i>Escherichia coli</i> and <i>Klebsiella pneumoniae</i> (first isolates* only for both) with a combination of cefuroxime and tobramycin	
5b	Empiric success rate for <i>Escherichia coli</i> and <i>Klebsiella pneumoniae</i> first isolates* only for both with a combination of amoxicillin & clavulanic acid and tobramycin OR amoxicillin & clavulanic acid and gentamicin	30
5c	Empiric success rate for <i>Escherichia coli</i> and <i>Klebsiella pneumoniae</i> first isolates* only for both with a combination of ceftazidime and tobramycin OR ceftazidime and gentamicin	

S = susceptible; I = susceptible, increased exposure; R = resistant

\*) Clinical and Laboratory Standards Institute, M39-A4 [13]

### 6.2.2 AMR data

Anonymised microbiological data were obtained from the Department of Medical Microbiology and Infection Prevention at the UMCG. The data consisted of 23,416 records from 18,508 unique blood culture tests that were taken between January 1, 2019 and December 31, 2019 which were retrieved from the local laboratory information system (LIS). Available variables were: test date, sample identification number, sample specimen, anonymised patient identification number, microbial identification code (if culture positive), antimicrobial susceptibility test results (S, I, R - susceptible, susceptible at increased exposure, resistant) for 52 antimicrobials. The exemplified data structure is presented in Table 2.

Table 2. Raw data example.

Patient ID	Date	Sample ID	Specimen	Mo	PEN	AMX	CXM
0001	2019-03-08	100	blood	esccol	R	I	S
0001	2019-03-09	101	blood	esccol	R	I	S
0002	2019-03-08	102	blood	staaur	R	S	-
0003	2019-03-08	103	blood	pse aer	R	R	R

S = susceptible; I = susceptible, increased exposure; R = resistant; Mo = microorganism; PEN = penicillin; AMX = amoxicillin; CXM = cefuroxime; esccol = *Escherichia coli*; staaur = *Staphylococcus aureus*; pse aer = *Pseudomonas aeruginosa*

### 6.2.3 AMR data analysis and reporting

We used our previously developed approach [7,8] to create a customised browser-based AMR data analysis and reporting application. This application was used in this study and applied to the AMR data analysis and reporting tasks listed in the task document (Table 1). The development of the application followed an agile approach using scrum methodologies [14]. Agile development was used to effectively and iteratively work in a team of two developers, a clinical microbiologist, and an infection preventionist. The application was designed as an interactive web-browser based dashboard (Figure 2). The prepared dataset was already loaded into the system and interaction with the application was possible through any web-browser.

### 6.2.4 Study participants

Participants in this study were recruited from the departments of Medical Microbiology, Critical Care Medicine, and Paediatrics, to reflect heterogeneous backgrounds of healthcare professionals working with AMR data. Members of the development team did not take part in the study.

### 6.2.5 Study execution and data

First, study participants were asked to fill in an online questionnaire capturing their personal backgrounds, demographics, software experience, and experience

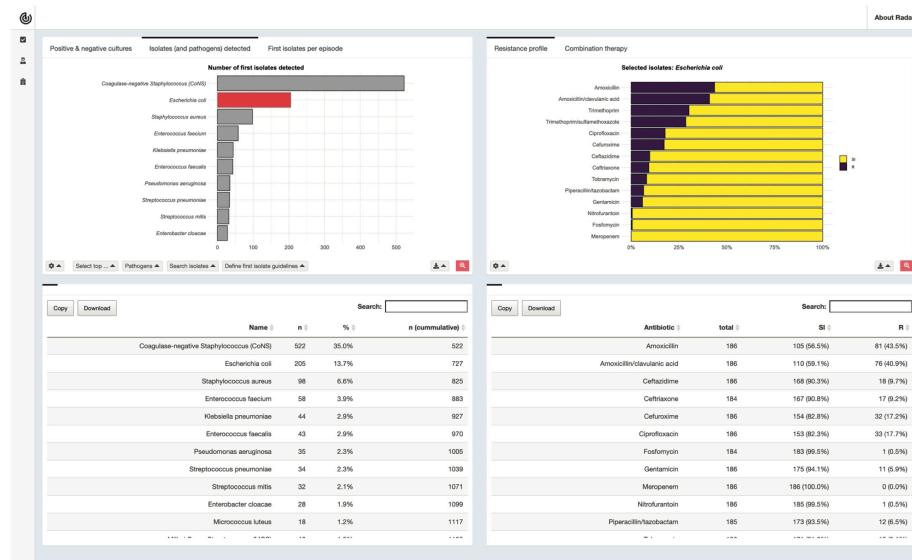


Figure 6.2: Interactive dashboard for AMR data analysis used in this study.

in AMR data analysis and reporting. Next, participants were provided the task document together with the AMR data (csv- or xlsx-format). The participants were asked to perform a comprehensive AMR data report following the task document using their software of choice (round 1). Task results and information on time spent per task were self-monitored and returned by the participant using a structured report form. Lastly, participants repeated the AMR data analysis and reporting process with the same task document but using the new AMR data analysis and reporting application (round 2). Task results and information on time spent per task were again self-monitored and returned by the participant using the same structured report form as in the first round. This last step was evaluated using a second online questionnaire. The study execution process is illustrated in Figure 1.

### 6.2.6 Evaluation and study data analysis

The utility of the new AMR data analysis and reporting application was evaluated according to ISO 9241-11:2018 [15]. This international standard comprises several specific metrics to quantify the usability of a tool with regard to reaching its defined goals (Figure 3). In this study the goal was a comprehensive AMR data report and comprised several tasks as outlined in the task document. The equipment was the focus of this study (traditional AMR data analysis and reporting approach vs. newly developed AMR data analysis and reporting approach).

The three ISO standard usability measures (in grey) were defined as follows in

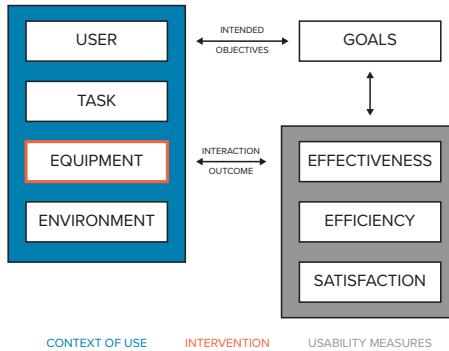


Figure 6.3: Usability framework based on ISO 9241-11.

this study: Effectiveness was determined by degree of task completion coded using three categories: 1) completed; 2) not completed (task not possible to complete); 3) not completed (task completion would take too long, e.g., > 20 minutes). In addition, effectiveness was assessed by the variance in the task results stratified by study round. Deviation from the correct results was measured in absolute percent from the correct result. To account for potential differences in the results due to rounding, all numeric results were transformed to integers. Efficiency was determined by timing each individual task. Time on task started when the user started performing the task, all data was loaded, and the chosen analysis software was up and running. Time on task ended when the task reached one of the endpoints, as described above. In the analysis, the mean time for each task and the mean total time for the complete report across users was calculated. Statistically significant difference was tested using paired Student's t-test. All analyses were performed in R [16]. Outcomes of tests were considered statistically significant for  $p < 0.05$ . Accuracy of the reported results per task and round were studied by calculating the deviation of the reported result in absolute percent from the correct result. Satisfaction was measured using the System Usability Scale (SUS), a 10-item Likert scale with levels from 1 (strongly disagree) to 5 (strongly agree, see Appendix A3) [17]. The SUS yields a single number from 0 to 100 representing a composite measure of the overall usability of the system being studied (SUS questions and score calculation in the Appendix A2).

## 6.3 Results

### 6.3.1 Study participants

In total 10 participants were recruited for this study. Most participants were clinical microbiologists (in training) (70%). The median age of the participant group was 40.5 years with a median working experience in the field of 8.0 years. The relevance of AMR data as part of the participants' job was rated very high

(median of 5.0; scale 1-5). AMR data analysis was part of the participants' job for 60% of all participants. Participants reported to be very experienced in interpreting AMR data structures (median 5.0, scale 1-5). Participants were less experienced in epidemiological data analysis (median 3.0, scale 1-5). All participant characteristics are summarised in Table 3.

Table 3. Study participant characteristics.

Characteristics	Overall (n=10)
<b>Age</b>	
Median [Min, Max]	40.5 [32.0, 61.0]
<b>Working experience in years</b>	
Median [Min, Max]	8.00 [1.00, 22.0]
<b>Job description</b>	
Infection preventionist	1 (10.0%)
Intensivist	1 (10.0%)
Clinical microbiologist	4 (40.0%)
Paediatrician	1 (10.0%)
Resident clinical microbiology	3 (30.0%)
<b>Worked with AMR data before</b>	
No	1 (10.0%)
Yes	9 (90.0%)
<b>Relevance of AMR data as part of the job</b>	
Scale: 1 = not relevant at all; 5 = very relevant	
Median [Min, Max]	5.00 [3.00, 5.00]
<b>AMR data analysis as part the job</b>	
No	4 (40.0%)
Yes	6 (60.0%)
<b>Familiarity with AMR data structure</b>	
Scale: 1 = not familiar at all; 5 = expert	
Median [Min, Max]	4.00 [1.00, 5.00]
Missing	1 (10.0%)
<b>Experience in interpreting AMR data (e.g., antibiograms)</b>	
Scale: 1 = no experience; 5 = very experienced	
Median [Min, Max]	5.00 [3.00, 5.00]
<b>Experience in epidemiological data analysis</b>	
Scale: 1 = no experience; 5 = very experienced	
Median [Min, Max]	3.00 [2.00, 5.00]
<b>Experience in working with AMR data</b>	
Scale: 1 = no experience; 5 = very experienced	
Median [Min, Max]	3.50 [1.00, 5.00]

The participants reported a diverse background in software experience for data analysis, with most experience reported for Microsoft Excel (Figure 4).

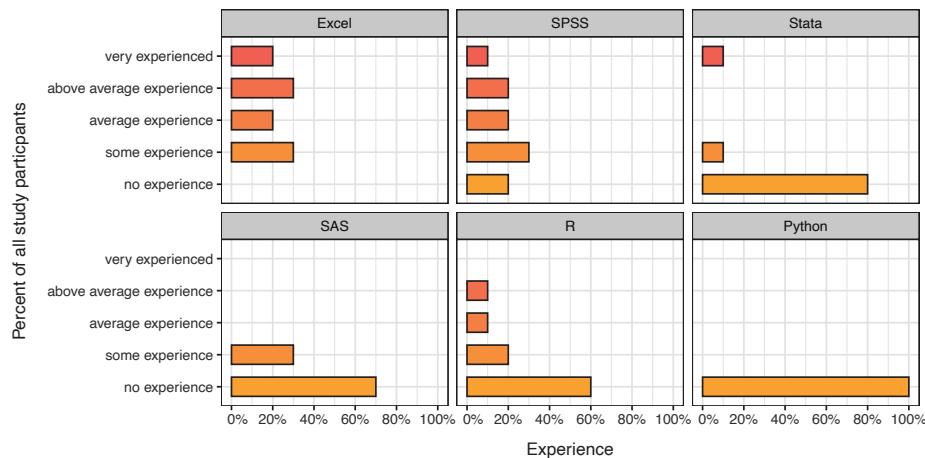


Figure 6.4: Data analysis software experience reported by study participants.

### 6.3.2 Effectiveness and accuracy

Not all participants were able to complete the tasks within the given time frame. Average task completion between the first round (traditional AMR data analysis and reporting) and the second round (new AMR data analysis and reporting) changed from 56% (SD: 23%) to 96% (SD: 6%) ( $p < 0.05$ ). Task completion per question and round is displayed in Figure 5. Variation in responses for each given task showed significant differences between the first and second round.

Figure 6 shows the deviation in absolute percent from the correct results from the correct result per round and task. The proportion of correct answers in the available results increased from 38% in the first round to 98% in the second round ( $p < 0.001$ ). A sub-analysis of species-specific results for task 3 round 1 is available in the appendix (A3).

### 6.3.3 Efficiency

Overall, the mean time spent per round was significantly reduced from 93.7 (SD: 21.6) minutes to 22.4 (SD: 13.7) minutes ( $p < 0.001$ ). Significant time reduction could be observed for tasks 2-5 (Figure 7). Analyses were further stratified to compare efficiency between participants that reported AMR data analysis as part of their job versus not part of the job. No significant time difference for completing all tasks could be found between the groups. However, in both groups the overall time for all tasks significantly decreased between the first and second round: on average by 70.7 minutes ( $p < 0.001$ ) in the group reporting AMR data analysis as part of their job and by 72.1 minutes ( $p = 0.01$ ) in the group not reporting AMR data analysis as part of their job.

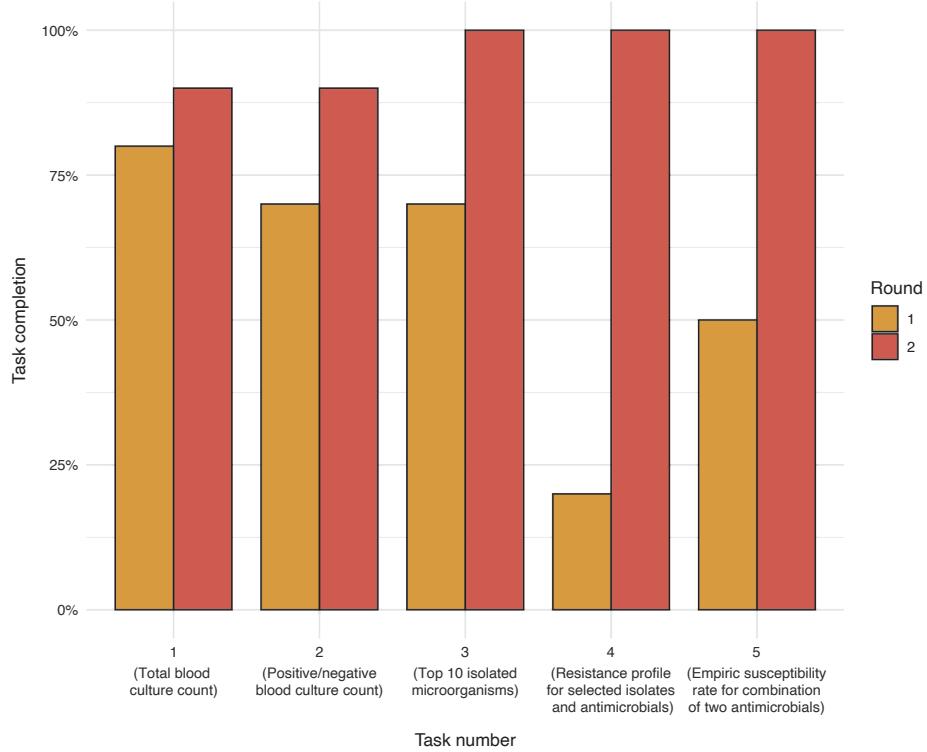


Figure 6.5: Task completion in percent by task number and round.

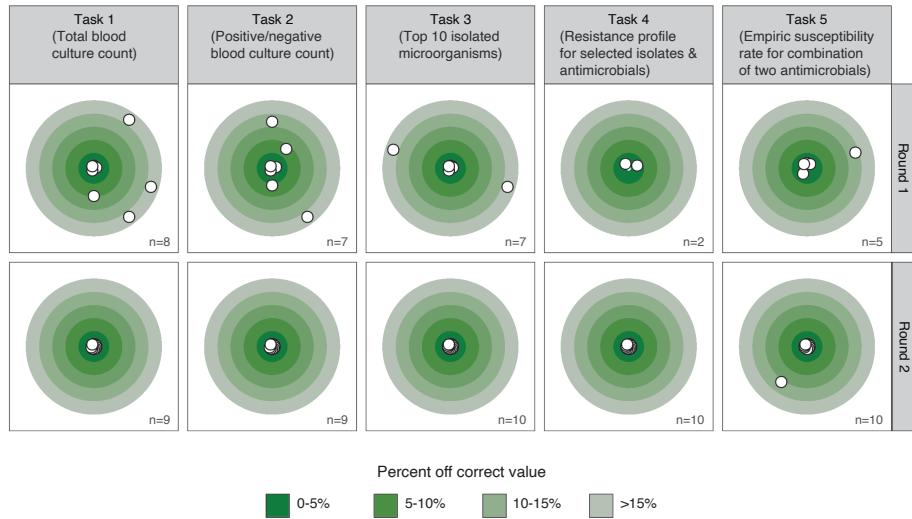


Figure 6.6: Deviation from the correct result by task and round in absolute percent from correct result. Only completed tasks (n) are shown.

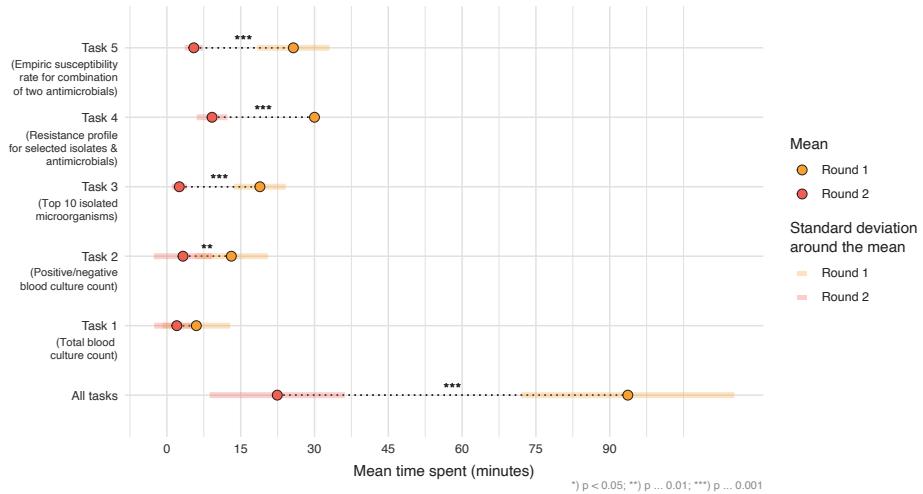


Figure 6.7: Mean time spent per task in minutes in each round (yellow = first round, red = second round). Statistical significance was tested using two-sided paired t-tests. All results were included irrespective of correctness of the results.

### 6.3.4 Satisfaction

Participants rated the usability of the new AMR reporting tool using the system usability scale (SUS) which takes values from 0 to 100 (Appendix A2). This resulted in a median of 83.8 on the SUS.

## 6.4 Discussion

This study demonstrates the effectiveness, efficiency, and accuracy of using open-source software tools to improve AMR data analysis and reporting. We applied our previously developed approach to AMR data analysis and reporting [7,8] in a clinical scenario and tested these tools with study participants (users) working in the field of AMR. Comparing traditional reporting tools with our newly developed reporting tools in a two-step process, we demonstrated the usability and validity of our approach. Based on a five item AMR data analysis and reporting task list and the provided AMR data, study participants reported significantly less time spent on creating an AMR data report (on average 93.7 minutes vs. 22.4 minutes;  $p < 0.01$ ). Task completion increased significantly from 56% to 96%, which indicates that with traditional reporting approaches common questions around AMR are hard to answer in a limited time. The accuracy of the results greatly improved using the new AMR reporting approach, implicating that erroneous answers are more common when users rely on general non-AMR-specific traditional software solutions. The usability of our AMR reporting approach was rated with a median of 83.8 on the SUS. The SUS is

widely used in usability assessments of software solutions. A systematic analysis of more than 1000 reported SUS scores for web-based applications across different fields has found a mean SUS score of 68.1 [18]. The results thus demonstrate a good usability of our approach.

The task list used in this study reflects standard AMR reporting tasks. More sophisticated tasks, such as the detection of multi-drug resistance according to (inter-)national guidelines were not included. However, these analyses are vital in any setting but restrained since the required guidelines are not included in traditional reporting and analysis tools (e.g., Microsoft Excel, SPSS, etc.). Notably, the underlying software used in this study [7] does provide methods to easily incorporate (inter)national guidelines such as the definitions for (multi-)drug resistance and country-specific (multi-)drug resistant organisms. The increase in task completion rate and accuracy of the results demonstrated that our tools empower specialists in the AMR field to generate reliable and valid AMR data reports. This is important as it enables detailed insights into the state of AMR on any level. These insights are often lacking. Our approach could fill this gap by democratising the ability for reliable and valid AMR data analysis and reporting.

This need is exemplified in the worrisome heterogeneity of the reporting results using traditional AMR reporting tools in the first round. Only 37.9% of the results in the first round were correct. Together with a task completion rate of 56%, this demonstrates that traditional tools are not suitable for AMR reporting. The inability of working in reproducible and transparent workflows further aggravates reporting with these traditional tools. All participants in the study should be able to produce standard AMR reports and 90% indicated that they worked with AMR data before. Sixty percent reported AMR data analysis to be part of their job, but no efficiency difference between groups were found. Our results show that AMR data analysis and reporting is challenging and can be highly error-prone. But an approach such as the one we developed can lead to correct results in a short time while being reproducible and transparent.

We chose an agile workflow which enabled us to integrate clinical feedback throughout the development process in this study. We can highly recommend this efficient approach for projects that need to bridge clinical requirements, statistical approaches, and software development. Our approach was inspired by others not in the AMR field that describe the use of reproducible open-source workflows in ecology [19]. We found that open-source software enables the transferability of methodological approaches across research fields. This transfer is a great example of the strength in the scientific community when working interdisciplinarily and sharing reliable and reproducible workflow.

This study is subject to limitations. Only ten participants were recruited for this study. Although low participant numbers are frequently observed in usability studies and reports show that only five participants suffice to study the usability of a new system, a larger sample size would be desirable [20–24]. In addition, other methods (e.g., ‘think aloud’ method) beyond the single use of the SUS for

the evaluation of our approach would further improve insights in the usability but were not possible in the study setting [25]. Although the introduction of new AMR data and reporting tools made use of an already available approach, implementation still requires staff experienced in R. Reporting requirements also differ per setting and tailor-made solutions incorporating different requirements are needed.

The present study shows that answering common AMR-related questions is tremendously burdened for professionals working with data. However, answers to such questions are the requirement to enable hospital-wide monitoring of AMR levels. The monitoring, be it on the institutional, regional, or (inter-) national level, can lead to alteration of treatment policies. It is thus of utmost importance that reliable results of AMR data analyses are ensured to avoid imprecise and erroneous results that could potentially be harmful to patients. We show that traditional reporting tools and applications that are not equipped for conducting microbiology epidemiological analyses seem unfit for this task - even for the most basic AMR data analyses. To fill this gap, we have developed new tools for AMR data analysis and reporting. In this study, we demonstrated that these tools can be used for better AMR data analysis and reporting in less time.

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## Conflict of interest

The authors report no conflict of interests.

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## Appendix

### Appendix A1: Task lists including correct results

Table A1. AMR data analysis and reporting tasks with correct results

Task	Task description	Correct result	
1	Total number of blood culture sets per year	18,468	
2a	Total number of positive blood culture sets per year	2,473	
2b	Total number of negative blood culture sets per year	15,995	
3	Top ten isolated microorganisms in blood cultures per year including isolate count (first isolates)		
	CoNS	522	
	<i>Escherichia coli</i>	205	
	<i>Staphylococcus aureus</i>	98	
	<i>Enterococcus faecium</i>	58	
	<i>Klebsiella pneumoniae</i>	44	
	<i>Enterococcus faecalis</i>	43	
	<i>Pseudomonas aeruginosa</i>	35	
	<i>Streptococcus pneumoniae</i>	34	
	<i>Streptococcus mitis</i>	32	
	<i>Enterobacter cloacae</i>	28	
4	Resistance profile (S/I & R) in selected isolates (first isolates) found in blood cultures for selected antimicrobials		
A	<i>Escherichia coli</i>	S/I (%)	R (%)
	Amoxicillin	56.4	43.5
	Amoxicillin / clavulanic acid	59.1	40.9
	Piperacillin / tazobactam	93.5	6.5
	Cefuroxime	82.8	17.2
	Ceftriaxone	90.8	9.2
	Ceftazidime	90.3	9.7
	Meropenem	100.0	0.0
	Ciprofloxacin	82.3	17.7
	Gentamicin	94.1	5.9
	Tobramycin	91.9	8.1
	Fosfomycin	99.5	0.5
	Trimethoprim	69.9	30.1
	Trimethoprim/sulfamethoxazole	71.5	28.5
	Nitrofurantoin	99.5	0.5
B	<i>Klebsiella pneumoniae</i>	S/I (%)	R (%)
	Amoxicillin	0.0	100.0

	Amoxicillin / clavulanic acid	75.6	24.4
	Piperacillin / tazobactam	87.8	12.2
	Cefuroxime	82.9	17.1
	Ceftriaxone	85.4	14.6
	Ceftazidime	85.4	14.6
	Meropenem	100.0	0.0
	Ciprofloxacin	87.8	12.2
	Gentamicin	97.6	2.4
	Tobramycin	92.7	7.3
	Fosfomycin	80.5	19.5
	Trimethoprim	85.4	14.6
	Trimethoprim / sulfamethoxazole	90.2	9.8
	Nitrofurantoin	0.0	100.0
<b>C</b>	<i>Staphylococcus aureus</i>	S/I (%)	R (%)
	Penicillin	24.7	75.3
	Flucloxacillin	96.6	3.4
	Gentamicin	97.7	2.3
	Erythromycin	85.4	14.6
	Clindamycin	89.9	10.1
	Doxycycline	97.8	2.2
	Linezolid	100.0	0.0
	Trimethoprim / sulfamethoxazole	94.4	5.6
	Rifampicin	98.9	1.1
<b>5</b>	Empiric susceptibility rate for selected isolates (first isolates) found in blood cultures with a combination of selected antimicrobials	S/I (%)	
<b>A</b>	<i>Escherichia coli</i>		
	Cefuroxime & tobramycin	96.8	
	<i>Klebsiella pneumoniae</i>		
	Cefuroxime & tobramycin	92.7	
<b>B</b>	<i>Escherichia coli</i>		
	Amoxicillin / clavulanic acid & tobramycin	93.0	
	Amoxicillin / clavulanic acid & gentamicin	95.2	
	<i>Klebsiella pneumoniae</i>		
	Amoxicillin / clavulanic acid & tobramycin	92.7	
	Amoxicillin / clavulanic acid & gentamicin	97.6	
<b>C</b>	<i>Escherichia coli</i>		
	Ceftriaxone & tobramycin	97.3	
	Ceftriaxone & gentamicin	98.4	

<i>Klebsiella pneumoniae</i>		
Ceftriaxone & tobramycin		92.7
Ceftriaxone & gentamicin		97.6

## Appendix A2: System Usability Scale (SUS)

1. I think that I would like to use this system frequently.
2. I found the system unnecessarily complex.
3. I thought the system was easy to use
4. I think that I would need the support of a technical person to be able to use this system.
5. I found the various functions in this system were well integrated.
6. I thought there was too much inconsistency in this system.
7. I would imagine that most people would learn to use this system very quickly.
8. I found the system very cumbersome to use.
9. I felt very confident using the system.
10. I needed to learn a lot of things before I could get going with this system.

(Each item with levels: 1 = strongly disagrees to 5 = strongly agrees)

Scores for individual items are not meaningful on their own. To calculate the SUS score, the score contributions from each item must be summed. Each item's score contribution ranges from 0 to 4. For items 1, 3, 5, 7, and 9 the score contribution is the scale position minus 1. For items 2, 4, 6, 8, and 10, the contribution is 5 minus the scale position. The sum of the scores is multiplied by 2.5 to obtain the SUS.

## Appendix A3: Task 3 sub-analysis

Task 3 asked participants to identify the ten most frequent species in the provided data set, while correcting for multiple occurrences of a species within a patient. Figure A3 illustrates the deviation from the correct result in the first round (traditional AMR reporting) per species. For this analysis also incomplete results were included (i.e., task not completed but some results provided).

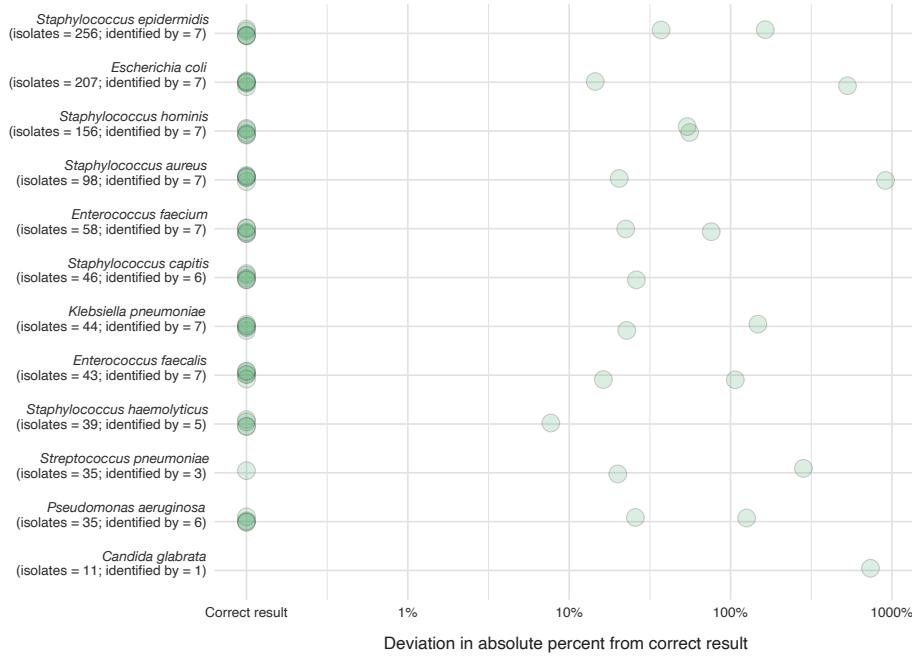


Figure 6.8: Results from task 3 in round 1. Deviation in absolute percent from the correct result per identified species. Also, incomplete data from participants was used in this analysis (i.e., task not completed but some results given). The correct number per species is given in addition to the number provided answers.



## **Chapter 7**

# **Trends in Occurrence and Phenotypic Resistance of Coagulase-Negative Staphylococci (CoNS) Found in Blood in the Northern Netherlands between 2013 and 2019**

In preparation

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## 7.1 Abstract

For years, coagulase-negative staphylococci (CoNS) were not considered a cause of bloodstream infections (BSIs) and were often regarded as contamination. However, the association of CoNS with nosocomial infections is increasingly recognised in research and clinical practice. At present, the CoNS group consists of 45 different species. Their identification has mainly been driven by the introduction of matrix-assisted laser desorption/ionisation time-of-flight (MALDI-TOF) mass spectrometry. Yet, treatment guidelines consider CoNS as a whole group and rarely differentiate between species, despite increasing antibiotic resistance (ABR) in CoNS. Therefore, this retrospective study provides an in-depth analysis of CoNS isolates and their ABR profiles found in blood culture isolates between 2013 and 2019 in a novel full-region approach including the entire region of the Northern Netherlands. In total, 10,796 patients were included that were hospitalised in one of the 15 hospitals in the region leading to a sample of 14,992 first CoNS isolates for (ABR) data analysis. CoNS accounted for 27.6% of all available 71,632 blood culture isolates. EUCAST Expert rules were applied to correct for errors in antibiotic test results. A total of 27 different species were found. Major differences were observed in the occurrence and ABR profiles of the different species. The top five species covered 97.1% of all included isolates: *S. epidermidis* (48.4%), *S. hominis* (33.6%), *S. capitis* (9.3%), *S. haemolyticus* (4.1%), and *S. warneri* (1.7%). Regarding ABR, *S. epidermidis* and *S. haemolyticus* showed 50-80% resistance to teicoplanin and macrolides while resistance to these agents remained lower than 10% in most other CoNS species. Yet, such differences are neglected in national guideline development causing a focus on 'ABR-safe' agents such as glycopeptides. Nonetheless, other agents could be considered viable options for some species where ABR never surpassed 10%. In conclusion, a multi-year, full-region approach to extensively assess the trends in both the occurrence and AMR of CoNS species was carried out which could be used for evaluating treatment policies and understanding more about these important but still too often neglected pathogens.

## 7.2 Introduction

Sepsis is a syndrome of physiologic, pathologic, and biochemical abnormalities induced by bloodstream infections (BSIs). It is the most frequent cause of death in hospitalised patients and has been recognised by the WHO as a global health priority [1,2]. For years, coagulase-negative staphylococci (CoNS) were not considered a cause of BSIs and were often regarded as contamination [3]. Yet, it has been shown that CoNS can cause BSIs and a high mortality rate [4,5], especially in immunocompromised patients and newborns [6,7]. Moreover, CoNS have become increasingly associated with nosocomial infections [8]. This is attributed

to (i) an increase of multimorbid and immunocompromised patients that are more prone to infections, (ii) the increased use of inserted foreign body material in modern medicine, and (iii) the property of CoNS to adapt molecularly to the hospital environment by diverging into new strains [8,9]. Specifically, *S. epidermidis* and *S. haemolyticus* are associated with sepsis caused by foreign-body-related infections (FBRIs), such as central line-associated BSIs and prosthetic joint infections [10].

At present, the CoNS group consists of 45 different species [11]. This group is highly heterogeneous in its prevalence in humans and, more importantly, its antibiotic resistance (ABR) patterns. Zooming in on CoNS at the species level is therefore useful to evaluate treatment options for CoNS causing BSI. The clinical interpretation and relevance of BSIs caused by CoNS are dependent on the determination at the species level, since not all species in the CoNS group are pathogenic and associated with sepsis or (other) nosocomial infections [8,12]. While the microbiological diagnosis of BSIs has for decades been based on blood samples cultivated in automated blood-culture systems, molecular and mass spectrometry (MS) approaches enable more reliable microbiological diagnosis [13,14]. Since 2012, matrix-assisted laser desorption/ionisation time-of-flight (MALDI-TOF) MS has become a standard for the identification of bacterial species and has, together with sequencing approaches, led to a rapid discovery of new species compared to formerly used techniques [15,16]. Prior to the use of MALDI-TOF MS, identification of CoNS was primarily performed with biochemical and physiological tests, which yielded variable results, particularly in less prevalent species [16]. Examples include *S. warneri*, *S. auricularis*, *S. capitis*, and other CoNS species that primarily colonise the skin of animals or are found on food products [17]. Due to less specific traditional test techniques, previously reported prevalences and ABR patterns of specific species in the CoNS group may have been unreliable or under-evaluated. Consequently, identification using MALDI-TOF MS has become crucial to analyse species-specific ABR.

ABR is a global healthcare problem and of great concern in the antibiotic therapy of BSIs. This also applies to the CoNS group where multi-drug resistance is common in species circulating in hospitals [18]. The rise of beta-lactam resistance in CoNS species has led to vancomycin as a first-line therapy against CoNS-mediated BSI in many countries, even though information about the pharmacokinetics and pharmacodynamics (PK/PD) of vancomycin against CoNS is limited [5,19–21]. To assess the constant change of ABR in CoNS, geo-spatial and temporal analyses of ABR are required.

In the Netherlands, country-wide ABR analyses are used to develop antibiotic treatment guidelines by the Dutch Working Party on Antibiotic Policy (Stichting Werkgroep Antibiotica Beleid, SWAB) [21,22]. Their recommendations are based on NethMap, an annually released national report about ABR and antibiotic consumption by the Dutch National Institute for Public Health and the Environment (Rijksinstituut voor Volksgezondheid en Milieu, RIVM) [21]. However, this national report does not specify nor address ABR on a patient,

hospital, or regional level.

Therefore, to inform clinical decision-makers this cross-sectional retrospective study provides an in-depth ABR analysis of all CoNS isolates found in blood cultures from 2013 until 2019 in the Northern Netherlands that were determined by MALDI-TOF MS. We aim to evaluate the differences in the occurrence of CoNS species and their ABR patterns and to assess their clinical microbiological relevance using a full-region approach.

## 7.3 Materials & methods

### 7.3.1 Study setting and patient cohort

This study was performed within the Northern Netherlands (Figure 1), a geographic region with 1.7 million inhabitants [23]. Its three provinces are similar in population density: Drenthe (492,167 inhabitants, 184/km<sup>2</sup>), Friesland (647,672 inhabitants, 183/km<sup>2</sup>) and Groningen (583,990 inhabitants, 243/km<sup>2</sup>) [23]. The study population consisted of 10,786 patients hospitalised with suspected BSI in 15 participating hospitals (14 secondary care, one tertiary care) located within this region between 1 January 2013 and 31 December 2019. All hospitals included at least one intensive care unit (ICU). There was no age restriction on including patients.

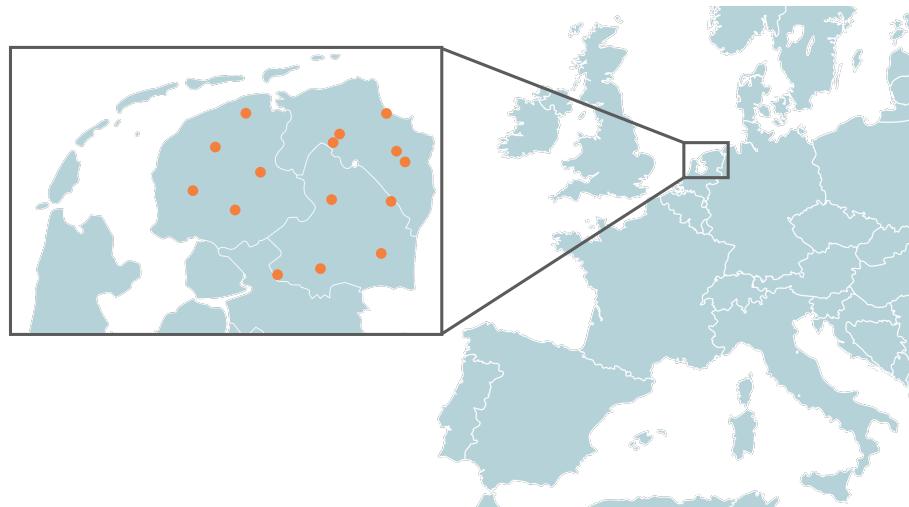


Figure 7.1: Locations of the fifteen hospitals in the three provinces in the North of the Netherlands. Between 2013 and June 2018, the region comprised fourteen hospitals; in July 2018, two hospitals merged into one new hospital, leaving a total of thirteen currently active hospitals.

### 7.3.2 Microbiological and demographic data

All blood cultures were routinely drawn and analysed at one of the three medical microbiological laboratories in the region (Izore, Friesland; Certe, Groningen and Drenthe; University Medical Center Groningen). After routine processing, isolates were included in the study if the species was characterised as a member of the CoNS group and antibiotic test results were available. In the study period, CoNS species were the most prevalent microorganisms isolated from blood and accounted for 27.6% of all available 71,632 blood culture isolates. The following variables were available for all isolates: date, name of laboratory, name of the hospital, age, gender, and ID of the patient and type of ward (ICU, clinical, outward). Genotypic data was not available for this study, as genotyping was not part of routine analysis.

### 7.3.3 Species determination and antibiotic susceptibility testing (AST)

Routine processing in the laboratories included the incubation of blood cultures allowing the colourimetric detection of CO<sub>2</sub> produced by growing microorganisms. Determination of the taxonomic species level was done using MALDI-TOF MS. Two laboratories cultivated blood samples using the BacT/ALERT system (bioMérieux, France) and identified bacterial strains using the VITEK MS system (bioMérieux, France). One laboratory cultivated blood samples using the BACTEC (Becton Dickinson, UK) and identified bacterial strains using the Microflex System (Bruker Corporation, USA). Since the databases of these proprietary systems are not publicly available, a qualitative assessment could not be attained, nor was this available in public literature.

AST was performed using the VITEK 2 Advanced Expert System after isolates were incubated on blood agar plates containing 5% sheep blood (BA+5%SB). Two laboratories used the VITEK 2 P-586 cartridges and one laboratory used the VITEK 2 P-657 cartridge which are both developed specifically for Gram-positive bacteria such as staphylococci. All results were authorised and validated by at least two laboratory technicians and one clinical microbiologist. Since different VITEK 2 cartridges were used, not all isolates were tested for all antibiotics analysed in this study. Supplementary Material 2 contains a full list of all included isolates and their respective AST results.

### 7.3.4 Selection of bacterial isolates

First isolates were determined and selected using the AMR package for R to exclude duplicate findings following the M39-A4 guideline by the Clinical Laboratory Standards Institute (CLSI) [24,25]. This guideline defines first isolates based on the species level per patient episode, regardless of body site and other phenotypical characteristics. The episode length for this study was defined as 365 days, resulting in the inclusion of a unique species once a year per patient.

In this study, several additions were made in extension to the CLSI guideline. As the CLSI guideline only considers the genus/species per episode, we investigated the added value to include changes in the ABR profile per genus/species and episode. For this purpose, we weighted the ABR profile of six preselected antibiotics, which were specifically chosen based on clinical relevance for Gram-positive bacteria, such as CoNS: erythromycin, oxacillin, rifampicin, teicoplanin, tetracycline, and vancomycin. Any change in these antibiotics from susceptible to resistant or vice-versa within the same species in the same patient within one episode was considered a ‘first weighted isolate’. ABR analysis results per species were included if at least 30 first isolates were available following the current CLSI guideline [24].

### 7.3.5 EUCAST rules and antibiotic resistance analysis

European Committee on Antimicrobial Susceptibility Testing (EUCAST) rules were applied to the AST results including EUCAST Expert Rules (v3.1, 2016), EUCAST Clinical Breakpoint Interpretations (v10.0, 2020), and EUCAST rules for Intrinsic Resistance and Unusual Phenotypes [26,27]. All applied changes can be found in Supplementary Table 1. Resistance was defined as the number of isolates with an antibiotic interpretation of R (resistant) divided by the total number of susceptible (S or I) isolates, following the latest EUCAST guideline [27].

### 7.3.6 Statistical analysis

All statistical analyses were done using R v4.0.3, RStudio v1.4, and the AMR package v1.6.0 [25,28]. To test for linear trends, linear regression analyses were performed. Contingency tables were tested with Fisher’s exact test when the size was 2x2 and Chi-squared tests otherwise. For likelihood ratio tests exact binomial tests were used. Outcomes of statistical tests were considered significant when  $p < 0.05$ .

### 7.3.7 Ethical considerations

Ethical approval and informed consent were not required according to the medical ethical committee of the University Medical Center Groningen (METc M21.277097). All data were anonymised at the associated laboratories before analysis.

## 7.4 Results

### 7.4.1 Patients and included isolates

A total of 10,796 patients were included in this seven-year study. The median age was 67 (IQR: 52-78) and 46.7% ( $n = 5,040$ ) of the patients was female. A total of 19,803 CoNS isolates were included, of which 14,992 isolates were used

for ABR analysis based on the “first weighted isolates” algorithm. A selection of first isolates using solely the CLSI guideline [24] would have yielded 12,971 isolates (-13.5%,  $p < 0.001$ ). On ICUs, 25.7% of the first weighted isolates was found in males compared to 17.0% in females ( $p < 0.001$ ). The number of ICU patients with CoNS compared to non-ICU patient with CoNS showed a significant difference between secondary care (17.5%, n = 1,403) and tertiary care (24.4%, n = 670,  $p < 0.001$ ). Yet, no significant difference was observed in the number of CoNS isolates found in ICU patients between secondary care (21.0%, n = 2,191) and tertiary care (22.8%, n = 1,034).

Table 1. Numbers and characteristics per gender of included patients of the included CoNS isolates.

	Female	Male	Total	<i>p</i> -value
Number of patients	5,040 (46.7%)	5,746 (53.3%)	10,786	<0.001
Median age	68 (IQR: 52-79)	67 (IQR: 52-77)	67 (IQR: 52-78)	<0.001
Total number of isolates	8,794 (44.4%)	11,009 (55.6%)	19,803	<0.001
Number of first isolates	6,026 (46.5%)	6,945 (53.5%)	12,971	<0.001
Number of first weighted isolates	6,887 (45.9%)	8,105 (54.1%)	14,992	<0.001

At total of 27 different species of the CoNS group were found within the isolate collection (Table 2). The top five species covered 97.1% (n = 14,560) of all first weighted isolates: *S. epidermidis* (n = 7,260, 48.4%), *S. hominis* (n = 5,033, 33.6%), *S. capitis* (n = 1,395, 9.3%), *S. haemolyticus* (n = 612, 4.1%), and *S. warneri* (n = 260, 1.7%).

The remaining 432 isolates (2.9%) consisted of: *S. lugdunensis* (n = 91, 0.6%), *S. saprophyticus* (n = 45, 0.3%), *S. pettenkoferi* (n = 44, 0.3%), *S. cohnii* (n = 43, 0.3%), *S. caprae* (n = 40, 0.2%), and 17 other species (n = 169, 1.1%).

Table 2. Overview of the total number of isolated CoNS species (not only first isolates) found between 2013 and 2019 in the Northern Netherlands.

#### 7.4.2 Occurrence of CoNS species

The occurrence of CoNS species was stratified by type of care, type of hospital ward, geographic province, gender, and age (Figure 2). Age was grouped into five groups: 0-11, 12-24, 25-54, 55-74, and 75 or more years. When stratifying by species level and the different types of care, the proportion of *S. epidermidis* among all CoNS isolates was 62.5% in tertiary care (n = 2,834) versus 42.3% in secondary care (n = 4,426;  $p = 0.049$ ). Overall, *S. hominis* was less frequent in tertiary care (20.3%, n = 919) than in secondary care (39.4%, n = 4,114,  $p = 0.013$ ), while the occurrence of other CoNS species was comparable between secondary and tertiary care. Yet, major differences in relative occurrence were observed between ICU and non-ICU status in secondary care. On secondary care ICUs, *S. epidermidis* accounted for 55.9% of all first

Microorganism	2013	2014	2015	2016	2017	2018	2019	Total
<i>S. arlettae</i>	-	-	1 (0.0%)	-	-	-	-	1 (0.0%)
<i>S. auricularis</i>	3 (0.2%)	8 (0.3%)	-	3 (0.1%)	7 (0.2%)	3 (0.1%)	6 (0.2%)	30 (0.2%)
<i>S. capitnis</i>	163 (8.2%)	211 (8.0%)	259 (9.1%)	235 (7.8%)	240 (8.1%)	262 (8.2%)	276 (8.8%)	1,646 (8.3%)
<i>S. caprae</i>	6 (0.3%)	5 (0.2%)	4 (0.1%)	16 (0.5%)	1 (0.0%)	11 (0.3%)	6 (0.2%)	49 (0.2%)
<i>S. carnosus</i>	1 (0.1%)	-	2 (0.1%)	-	-	-	-	3 (0.0%)
<i>S. chromogenes</i>	-	1 (0.0%)	-	-	2 (0.1%)	-	-	3 (0.0%)
<i>S. cohnii</i>	9 (0.5%)	6 (0.2%)	7 (0.2%)	11 (0.4%)	5 (0.2%)	5 (0.2%)	4 (0.1%)	47 (0.2%)
<i>S. condimenti</i>	-	-	-	-	-	2 (0.1%)	1 (0.0%)	3 (0.0%)
<i>S. epidermidis</i>	1,024 (51.5%)	1,365 (51.7%)	1,557 (54.5%)	1,546 (51.2%)	1,544 (51.9%)	1,606 (50.3%)	1,543 (49.2%)	10,185 (51.4%)
<i>S. equorum</i>	-	-	-	1 (0.0%)	3 (0.1%)	-	1 (0.0%)	5 (0.0%)
<i>S. gallinarum</i>	-	-	-	1 (0.0%)	-	-	-	1 (0.0%)
<i>S. haemolyticus</i>	72 (3.6%)	130 (4.9%)	141 (4.9%)	154 (5.1%)	145 (4.9%)	198 (6.2%)	141 (4.5%)	981 (5.0%)
<i>S. hominis</i>	631 (31.8%)	816 (30.9%)	789 (27.6%)	945 (31.3%)	934 (31.4%)	1,009 (31.6%)	1,037 (33.1%)	6,161 (31.1%)
<i>S. kloosii</i>	-	-	-	1 (0.0%)	-	-	-	1 (0.0%)
<i>S. lentus</i>	-	-	-	1 (0.0%)	-	1 (0.0%)	1 (0.0%)	3 (0.0%)
<i>S. lugdunensis</i>	27 (1.4%)	25 (0.9%)	11 (0.4%)	19 (0.6%)	17 (0.6%)	23 (0.7%)	31 (1.0%)	153 (0.8%)
<i>S. massiliensis</i>	-	4 (0.2%)	-	-	-	-	-	4 (0.0%)
<i>S. pasteurii</i>	3 (0.2%)	4 (0.2%)	6 (0.2%)	3 (0.1%)	3 (0.1%)	4 (0.1%)	9 (0.3%)	32 (0.2%)
<i>S. pettenkoferi</i>	3 (0.2%)	2 (0.1%)	3 (0.1%)	2 (0.1%)	2 (0.1%)	13 (0.4%)	22 (0.7%)	47 (0.2%)
<i>S. piscifermentans</i>	-	-	-	-	-	1 (0.0%)	2 (0.1%)	3 (0.0%)
<i>S. saccharolyticus</i>	-	1 (0.0%)	4 (0.1%)	1 (0.0%)	5 (0.2%)	2 (0.1%)	5 (0.2%)	18 (0.1%)
<i>S. saprophyticus</i>	2 (0.1%)	12 (0.5%)	7 (0.2%)	16 (0.5%)	10 (0.3%)	6 (0.2%)	3 (0.1%)	56 (0.3%)
<i>S. schleiferi</i>	7 (0.4%)	6 (0.2%)	17 (0.6%)	7 (0.2%)	4 (0.1%)	3 (0.1%)	4 (0.1%)	48 (0.2%)
<i>S. sciuri</i>	-	1 (0.0%)	1 (0.0%)	2 (0.1%)	-	-	-	4 (0.0%)
<i>S. simulans</i>	4 (0.2%)	1 (0.0%)	3 (0.1%)	4 (0.1%)	4 (0.1%)	6 (0.2%)	6 (0.2%)	28 (0.1%)
<i>S. warneri</i>	31 (1.6%)	39 (1.5%)	42 (1.5%)	49 (1.6%)	46 (1.5%)	38 (1.2%)	38 (1.2%)	283 (1.4%)
<i>S. xylosus</i>	1 (0.1%)	2 (0.1%)	1 (0.0%)	-	3 (0.1%)	-	1 (0.0%)	8 (0.0%)
<b>Total</b>	<b>1,987 (100%)</b>	<b>2,639 (100%)</b>	<b>2,855 (100%)</b>	<b>3,017 (100%)</b>	<b>2,975 (100%)</b>	<b>3,193 (100%)</b>	<b>3,137 (100%)</b>	<b>19,803 (100%)</b>

weighted CoNS isolates found while on non-ICU wards this was 39.1% ( $p < 0.001$ ). In contrast, *S. hominis* accounted for 25.7% on secondary care ICUs while on non-ICU wards this was 43.3% ( $p < 0.001$ ). Notably, *S. hominis* was found 105 times (7.53%) in children under the age of one.

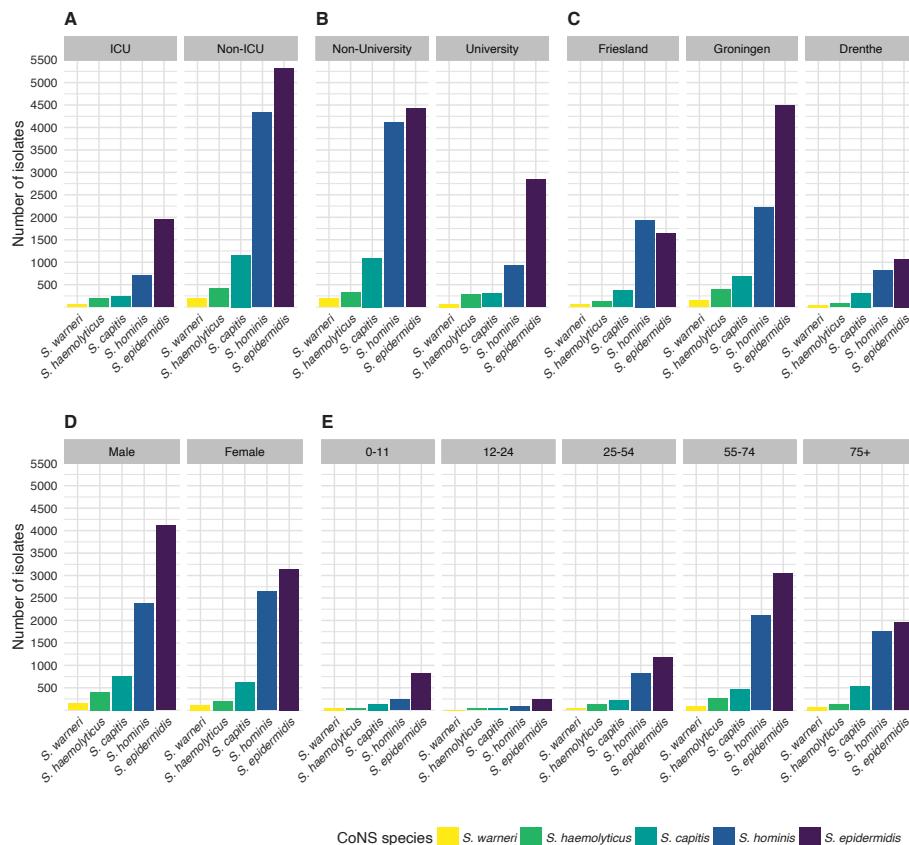


Figure 7.2: The number of first weighted isolates of the top five CoNS species found in the study stratified by (A) type of care, (B) type of hospital ward, (C) province of the Netherlands, (D), gender, and (E) age group.

Although all three provinces in the study region are similar in population density and gender distribution [23], major differences were observed in the occurrence of CoNS species between those provinces in secondary care. The occurrence of *S. epidermidis* among CoNS species in secondary care hospitals in Friesland was 38.7% in contrast to 43.7% and 45.9% in Drenthe and Groningen respectively ( $p < 0.001$ ). *S. hominis* was significantly more often found in secondary care hospitals in Friesland (45.9%) than in Drenthe (33.3%) and Groningen (36.0%) ( $p < 0.001$ ). Drenthe and Groningen did not differ significantly in the occurrence of CoNS species in secondary care.

Overall, there was no significant change in species distribution over the years. Stratified by gender, a linear increase of *S. hominis* over time ( $p = 0.001$ ) and a decrease of *S. epidermidis* ( $p = 0.005$ ) was found in males. In females, the occurrence of *S. hominis* also increased over time ( $p = 0.008$ ), but no decrease of *S. epidermidis* or any other species was observed. In age groups, no significant trends in occurrence were observed.

#### 7.4.3 Definition of CoNS persistence

In this retrospective study, it was impossible to differentiate between contaminated blood cultures and BSI-associated blood cultures, as clinical information was not available. Yet, to assess probable cases of BSIs caused by CoNS, we defined ‘CoNS persistence’ as a surrogate. CoNS persistence was defined by at least three positive blood cultures drawn on three different days within 60 days containing the same CoNS species within the same patient. In total, we identified 294 cases of CoNS persistence (Table 3). Aside from *S. massiliensis* that caused CoNS persistence in only one patient, the relatively most common causal agent of CoNS persistence was *S. haemolyticus* (5.8%,  $n = 32$ ,  $p < 0.001$ ), followed by *S. epidermidis* (3.7%,  $n = 212$ ,  $p < 0.001$ ), and *S. lugdunensis* (3.4%,  $n = 3$ ,  $p = 0.46$ ).

Table 3. The number of patients with and without CoNS persistence per species.

Microorganism	Patients with CoNS persistence	Patients without CoNS persistence	Total number of patients	Percentage of patients with CoNS persistence	Comparison with other species*
<i>S. massiliensis</i>	1	0	1	100%	N/A
<i>S. haemolyticus</i>	32	519	551	5.8%	$p < 0.001$
<i>S. epidermidis</i>	212	5,466	5,678	3.7%	$p < 0.001$
<i>S. lugdunensis</i>	3	86	89	3.4%	$p = 0.46$
<i>S. saprophyticus</i>	1	42	43	2.3%	$p = 1$
<i>S. hominis</i>	40	4,496	4,536	0.9%	$p < 0.001$
<i>S. capitis</i>	5	1,351	1,356	0.4%	$p < 0.001$
All other species	0	547	547	0%	N/A

N/A: not applicable (not all values >0). Please note that this table represents number of patients, not the number of isolates.

\* Fisher Exact Test on 2x2 contingency tables comparing number of patients with CoNS persistence with current species vs. all other species.

#### 7.4.4 Antibiotic resistance analysis

Clinically relevant antibiotics and their respective ABR profiles were analysed and compared for the top five CoNS species. Figure 3 shows time trends regarding the ABR profiles to ten different clinically relevant antibiotics, while Table 4 contains resistance percentages of all applicable combinations of species and antibiotic agents. In the following subsections, more detail on occurrence and trends is provided per antibiotic class based on Figure 3 and Table 4. Comprehensive ABR analyses per species of all available variables can be found in

Supplementary Table 3.

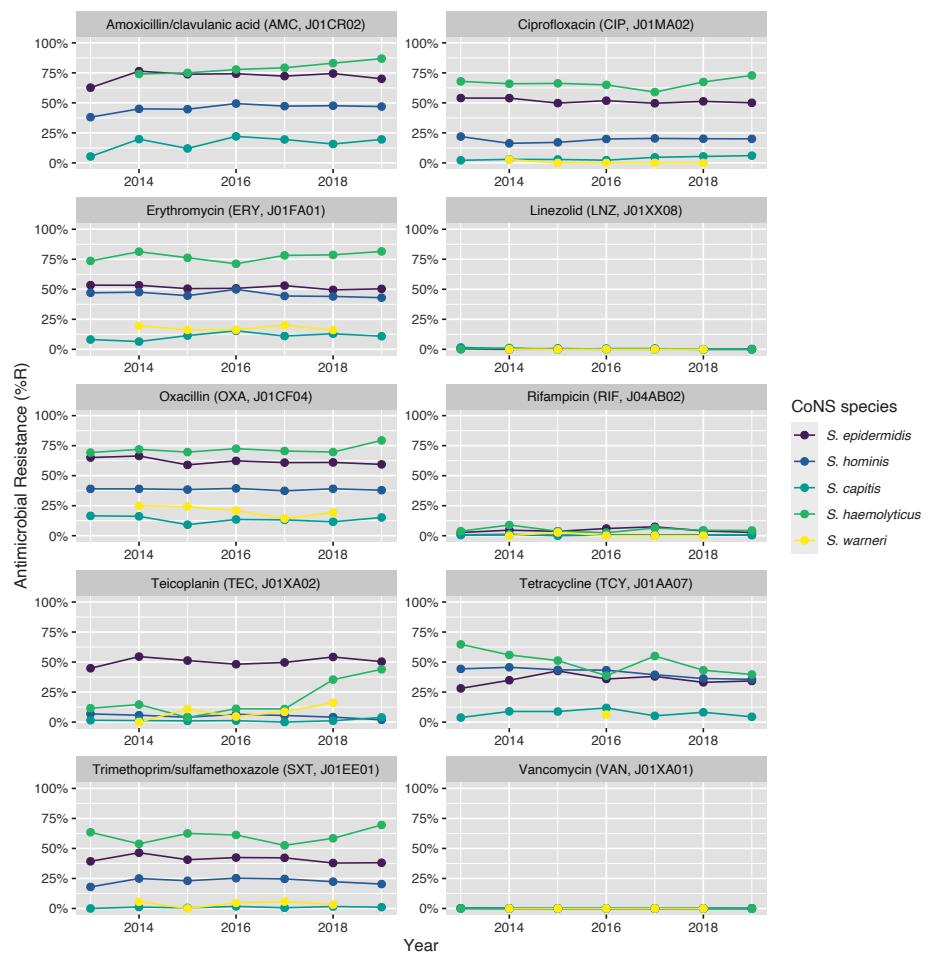


Figure 7.3: Antibiotic resistance of the five most frequent CoNS ( $n = 14,560$ ) over time between 2013 and 2019. Lines and points are missing where there were less than 30 isolates available for analysis.

Table 4. Antibiotic resistance in all first weighted CoNS isolates in blood between 2013 and 2019 where at least 30 isolates were available for ABR analysis. Resistance of 100% denotes intrinsic resistance, as defined by EUCAST. Between parentheses are the number of resistant first weighted isolates and the total number of first weighted isolates for that bug-drug combination. The antibiotic names are followed by the official EARS-Net code (European Antimicrobial Resistance Surveillance Network) and ATC code (Anatomical Therapeutic Chemical).

Antibiotic class	Antibiotic	<i>S. capitis</i>	<i>S. cohnii</i>	<i>S. epidermidis</i>	<i>S. haemolyticus</i>	<i>S. hominis</i>	<i>S. lugdunensis</i>	<i>S. pettenkoferi</i>	<i>S. saprophyticus</i>	<i>S. schleiferi</i>	<i>S. numeri</i>
Aminoglycosides	Gentamicin (CEN, [0]GBu3)	5.8% (7/123)	2.6% (1/39)	0.0% (0/38)	40.7% (2,744/6,739)	62.2% (352/561)	7.3% (33/453)	0.0% (0/37)	0.0% (0/39)	0.0% (0/34)	13.2% (34/228)
Kanamycin (KAN, [0]GBu4)	34.3% (79/230)	80.3% (3,174/3,954)	89.7% (358/399)	63.1% (1,167/6,866)	63.1% (355/563)	18.1% (816/4,518)	3.7% (3/82)	0.0% (0/35)	0.0% (0/38)	41.9% (31/74)	
Tobramycin (TOB, [0]GBu1)	6.6% (79/1,198)	2.9% (1/33)	47.4% (3,167/6,866)	0.4% (0/37)	1.6% (75/4,662)	2.7% (177/386)	0.0% (0/35)	0.0% (0/30)	0.0% (0/33)	13.0% (34/230)	
Chloramphenicol (CHL, [0]BA01)	0.4% (3/817)	0.0% (0/37)	0.0% (0/37)	0.0% (0/37)	0.0% (0/37)	0.0% (0/37)	0.0% (0/35)	0.0% (0/30)	0.0% (0/33)	0.6% (1/170)	
Rifampicin (RIF, [0]AB02)	0.6% (7/1,218)	0.0% (0/39)	0.0% (0/37)	0.0% (0/37)	0.0% (0/37)	0.0% (0/36)	0.0% (0/35)	0.0% (0/30)	0.0% (0/33)	0.4% (1/229)	
Antimycobacterials											
Beta-lactams/ penicillins	Amoxicillin (AMX, [0]CA04)	64.8% (107/165)	95.4% (3,026/3,173)	92.8% (280/299)	93.6% (1,065/1,448)	92.8% (280/299)	2.8% (25/549)	0.0% (0/82)	0.0% (0/39)	0.0% (0/34)	73.5% (36/49)
Amoxicillin/ clavulanic acid (AMC, [0]CR02)	16.6% (107/643)	16.6% (3,026/4,153)	72.9% (280/360)	77.8% (1,064/2,310)	46.1% (54/42)	11.9% (5/42)	0.0% (0/36)	0.0% (0/118)	0.0% (0/36)	30.5% (36/118)	
Ampicillin (AMP, [0]CA01)	64.8% (107/165)	95.4% (3,026/3,173)	92.8% (280/299)	93.6% (1,065/1,448)	92.8% (280/299)	2.8% (25/549)	0.0% (0/82)	0.0% (0/39)	0.0% (0/34)	73.5% (36/49)	
Benzylpenicillin (PEN, [0]CE01)	78.9% (870/1,102)	83.3% (30/36)	97.1% (34/35)	92.5% (5,927/6,411)	89.2% (486/545)	88.2% (3,714/4,209)	57.5% (427/73)	85.3% (20/34)	25.0% (8/32)	77.8% (161/207)	
Flucloxacillin (FLC, [0]CF45)	11.4% (140/1,227)	5.1% (21/398)	55.3% (4,144/6,009)	61.8% (46/504)	72.0% (1,684/4,229)	57.2% (61/82)	7.3% (40/56)	40.5% (15/37)	13.2% (5/38)	20.1% (46/229)	
Oxacillin (OXA, [0]CF04)	13.4% (160/1,197)	5.1% (22/34)	64.7% (4,135/6,677)	61.9% (493/559)	72.1% (1,492/4,386)	38.6% (61/82)	7.3% (14/35)	40.0% (14/35)	16.2% (6/37)	20.5% (47/229)	
Carbapenems											
Imipenem (IPM, [0]DH151)	21.8% (67/307)	54.3% (1,825/2,486)	73.4% (197/247)	79.8% (506/532)	54.3% (197/247)	54.3% (506/532)	0.0% (0/56)	0.0% (0/56)	28.6% (16/56)	28.6% (16/56)	
Meropenem (MEM, [0]DH02)	21.8% (67/307)	21.8% (1,823/2,485)	73.4% (197/247)	79.8% (506/532)	54.3% (197/247)	54.3% (506/532)	0.0% (0/56)	0.0% (0/56)	28.6% (16/56)	28.6% (16/56)	
Cephalosporins (1st gen.)	Cefazolin (CZO, [0]DBu4)	21.8% (67/307)	73.4% (1,823/2,484)	79.8% (197/247)	54.3% (506/532)	54.3% (506/532)	0.0% (0/56)	0.0% (0/56)	28.6% (16/56)	28.6% (16/56)	
Cephalosporins (2nd gen.)	Cefotaxim (COTX, [0]DD01)	21.8% (67/307)	73.4% (1,823/2,484)	79.8% (197/247)	54.3% (506/532)	54.3% (506/532)	0.0% (0/56)	0.0% (0/56)	28.6% (16/56)	28.6% (16/56)	
Cefuroxime (CXM, [0]DC02)	1.25% (104/831)	56.7% (17/30)	63.9% (3,025/4,333)	70.2% (280/399)	40.6% (1,063/2,211)	40.6% (1,063/2,211)	8.8% (5/57)	23.5% (36/153)	23.5% (36/153)	28.6% (16/56)	
Cefotaxime (CTX, [0]DD01)	21.8% (67/307)	73.4% (1,824/2,485)	79.8% (197/247)	54.3% (506/532)	54.3% (506/532)	54.3% (506/532)	0.0% (0/56)	0.0% (0/56)	28.6% (16/56)	28.6% (16/56)	
Ceftriaxone (CRO, [0]DD04)	21.8% (67/307)	73.4% (1,823/2,484)	79.8% (197/247)	54.3% (506/532)	54.3% (506/532)	54.3% (506/532)	0.0% (0/56)	0.0% (0/56)	28.6% (16/56)	28.6% (16/56)	
Cefoperazone (CPE, [0]DE01)	21.8% (67/307)	73.4% (1,823/2,484)	79.8% (197/247)	54.3% (506/532)	54.3% (506/532)	54.3% (506/532)	0.0% (0/56)	0.0% (0/56)	28.6% (16/56)	28.6% (16/56)	
Cephalosporins (4th gen.)	Cefepime (FEP, [0]DE01)	21.8% (67/307)	73.4% (1,823/2,484)	79.8% (197/247)	54.3% (506/532)	54.3% (506/532)	0.0% (0/56)	0.0% (0/56)	28.6% (16/56)	28.6% (16/56)	

Antibiotic class	Antibiotic	<i>S. apidis</i>	<i>S. aguae</i>	<i>S. colini</i>	<i>S. enteritidis</i>	<i>S. heidelberg</i>	<i>S. hominis</i>	<i>S. infantis</i>	<i>S. lactis</i>	<i>S. newport</i>	<i>S. schefußii</i>	<i>S. saprophyticus</i>	<i>S. typhimurium</i>	<i>S. weissmannii</i>	
Glycopeptides	Ticarcillin (TIC, J01XA02)	1.4% (17/1,190)	0.1% (0/39)	50.5% (0/35)	20.2% (27/132/5,448)	51.1% (0/5/520)	3.7% (3/82)	0.0% (0/51)	0.0% (0/38)	0.0% (0/38)	0.0% (0/38)	0.0% (0/38)	0.0% (0/38)	9.6% (22/230)	
Vancomycin (VAN, J01XA01)	0.0% (0/1,195)	0.1% (0/39)	0.1% (0/35)	0.1% (0/6,674)	0.1% (0/560)	0.0% (1/4,524)	0.0% (0/82)	0.0% (0/35)	0.0% (0/38)	0.0% (0/38)	0.0% (0/38)	0.0% (0/38)	0.0% (0/38)	0.0% (0/230)	
Macrolides/ lincomycins	Aztreonam (AZM, J01FA09)	11.0% (136/1,232)	12.8% (5/39)	44.7% (17/38)	51.5% (3,471/6,741)	77.6% (437/563)	45.7% (2,087/4,264)	12.2% (10/82)	12.2% (7/37)	12.2% (7/37)	12.2% (7/37)	12.2% (7/37)	12.2% (7/37)	12.2% (7/37)	17.5% (40/228)
Cephalosporins	Carbenicillin (CBL, J01FA09)	11.0% (136/1,231)	12.8% (5/39)	44.7% (17/38)	51.5% (3,471/6,741)	77.6% (437/563)	45.7% (2,086/4,263)	12.2% (10/82)	12.2% (7/37)	12.2% (7/37)	12.2% (7/37)	12.2% (7/37)	12.2% (7/37)	12.2% (7/37)	17.5% (40/228)
Other antibiotics	Cindamycin (CLL, J01FF01)	10.8% (132/1,224)	5.1% (2/39)	26.3% (10/38)	43.4% (2,910/6,706)	45.6% (233/555)	29.6% (1,347/4,547)	12.0% (10/83)	12.0% (10/83)	12.0% (10/83)	12.0% (10/83)	12.0% (10/83)	12.0% (10/83)	12.0% (10/83)	4.4% (16/229)
Fusidic acid (FRX, J01FA01)	Erythromycin (FRX, J01FA01)	11.0% (136/1,231)	12.8% (5/39)	44.7% (17/38)	51.5% (3,471/6,741)	77.6% (437/563)	45.7% (2,086/4,265)	12.2% (10/82)	12.2% (7/37)	12.2% (7/37)	12.2% (7/37)	12.2% (7/37)	12.2% (7/37)	12.2% (7/37)	17.5% (40/228)
Mupirocin (MLP, R01AX06)	2.6% (32/1,215)	2.6% (1/39)	0.0% (0/36)	14.8% (0/87)	3.7% (2/1,562)	6.5% (297/4,536)	0.0% (0/82)	0.0% (0/37)	0.0% (0/37)	0.0% (0/37)	0.0% (0/37)	0.0% (0/37)	0.0% (0/37)	1.7% (4/230)	
Nitrofuranation (NTR, J01XE01)	0.7% (2/289)	0.0% (0/36)	0.0% (0/33)	0.0% (0/33)	0.0% (0/1,732)	0.0% (0/160)	0.0% (8/926)	0.0% (0/926)	0.0% (0/926)	0.0% (0/926)	0.0% (0/926)	0.0% (0/926)	0.0% (0/926)	0.0% (0/926)	
Oxazolidinones	Lincosolid (LNZ, J01XX08)	0.4% (5/1,192)	0.0% (0/39)	0.0% (0/33)	0.1% (0/6,641)	0.2% (0/559)	0.0% (0/82)	0.0% (0/34)	0.0% (0/34)	0.0% (0/34)	0.0% (0/34)	0.0% (0/34)	0.0% (0/34)	0.0% (0/34)	
Quinolones	Ciprofloxacin (CPF, J01MA02)	3.8% (47/1,229)	2.6% (1/38)	0.0% (0/38)	51.5% (3,486/6,740)	66.4% (3,945/563)	19.4% (885/4,561)	4.8% (485/563)	8.1% (3/37)	8.1% (3/37)	8.1% (3/37)	8.1% (3/37)	8.1% (3/37)	0.4% (1/229)	
Moxifloxacin (MXF, J01MA14)	0.0% (0/396)	0.0% (0/396)	0.0% (0/33)	6.8% (0/33)	26.4% (88/1,202)	3.6% (24/91)	0.0% (45/1,239)	0.0% (0/30)	0.0% (0/30)	0.0% (0/30)	0.0% (0/30)	0.0% (0/30)	0.0% (0/30)	0.0% (0/30)	
Tetracyclines	Doxycycline (DOX, J01AM02)	5.9% (54/914)	5.9% (54/914)	32.9% (1,182/3,597)	46.0% (122/2,680)	34.0% (967/2,848)	14.5% (9/62)	0.0% (0/62)	0.0% (0/62)	0.0% (0/62)	0.0% (0/62)	0.0% (0/62)	0.0% (0/62)	3.5% (6/172)	
Minocycline (MINO, J01AM08)	0.0% (0/826)	0.0% (0/826)	0.0% (0/826)	0.0% (0/826)	0.0% (1/2,496)	0.3% (1/158)	0.0% (6/2,064)	0.0% (0/53)	0.0% (0/53)	0.0% (0/53)	0.0% (0/53)	0.0% (0/53)	0.0% (0/53)	0.0% (0/168)	
Tetracycline (TCY, J01AM07)	7.3% (71/971)	6.7% (2/30)	55.0% (1,521/4,347)	50.2% (103/3,325)	41.0% (1,544/3,765)	15.4% (10/65)	15.4% (10/65)	15.4% (10/65)	15.4% (10/65)	15.4% (10/65)	15.4% (10/65)	15.4% (10/65)	15.4% (10/65)	4.5% (8/176)	
Trimethoprim	Trimethoprim (TMP, J01EA01)	10.6% (36/339)	10.6% (36/339)	2.6% (1/38)	77.4% (2,923/3,775)	90.2% (3,981/3,888)	55.2% (1,364/2,470)	2.4% (2/82)	2.4% (2/82)	2.4% (2/82)	2.4% (2/82)	2.4% (2/82)	2.4% (2/82)	17.5% (10/57)	
Trimethoprim/ sulfamethoxazole	Trimethoprim/ sulfamethoxazole (SXT, J01EE01)	1.0% (12/1,233)	0.0% (0/39)	24.1% (1/38)	24.1% (2,764/6,735)	60.1% (338/562)	22.8% (1,037/4,256)	2.4% (2/82)	2.4% (2/82)	2.4% (2/82)	2.4% (2/82)	2.4% (2/82)	2.4% (2/82)	3.5% (8/229)	

#### 7.4.4.1 Glycopeptides

Vancomycin resistance was found in six *S. epidermidis* isolates (0.1%) and in one *S. hominis* isolate (0.0%). Half of all *S. epidermidis* isolates showed resistance to teicoplanin (50.5%, n = 2,752), which increased over the seven study years (min-max: 44.8%-54.5%, p = 0.001). An increase in teicoplanin resistance was observed in *S. haemolyticus* (min-max: 10.9%-44.0%, p < 0.001). Teicoplanin resistance remained low in *S. capitis* (1.4%, n = 17), *S. hominis* (5.1%, n = 202), and *S. warneri* (9.6%, n = 22).

#### 7.4.4.2 Macrolides

Erythromycin resistance was highest in *S. haemolyticus* (77.6%, n = 437), followed by in *S. epidermidis* (51.5%, n = 3,471), *S. hominis* (45.7%, n = 2,086), *S. warneri* (17.5%, n = 40), and *S. capitis* (11.0%, n = 136). Resistance to azithromycin and clarithromycin was equal to erythromycin resistance, due to EUCAST expert rules. However, resistance to clindamycin remained lower than resistance to erythromycin in all species: 45.6% (n = 253) in *S. haemolyticus* and 43.4% (n = 2,910) in *S. epidermidis*, 29.6% (n = 1,347) in *S. hominis*, 4.4% (n = 10) in *S. warneri*, and 10.8% (n = 132) in *S. capitis*.

#### 7.4.4.3 Fluoroquinolones

The highest ciprofloxacin resistance was found in *S. haemolyticus* (66.4%; n = 374) and *S. epidermidis* (51.5%; n = 3,468). Resistance to moxifloxacin was 26.4% (n = 24) in *S. haemolyticus* and less than 10% in all other species.

#### 7.4.4.4 Beta-lactams/penicillins

Oxacillin resistance was as high as 61.9% (n = 4,135) in *S. epidermidis*, which was thus the proportion of MRSE (methicillin-resistant *S. epidermidis*) among all *S. epidermidis* isolates in this study. Oxacillin resistance in *S. haemolyticus* was even higher (72.1%, n = 403) but considerably lower in all other CoNS species (13.4%-38.6%). Almost all *S. epidermidis*, *S. haemolyticus*, and *S. hominis* were resistant to amoxicillin (95.4%, 93.6%, and 92.8% respectively), while all other species showed amoxicillin resistance ranging between 64.8% and 73.5%. Resistance to amoxicillin/clavulanic acid was 72.9% (n = 3,026) in *S. epidermidis*. *S. haemolyticus* showed a strong linear increase in amoxicillin/clavulanic acid resistance (p < 0.001) since 2013 with 87% resistance in 2019 (n = 61).

#### 7.4.4.5 Other antibiotics

Resistance remained low to rifampicin in *S. haemolyticus* (5.0%; n = 28) and *S. epidermidis* (4.5%; n = 300) and remained less than 0.6% in all other species. Linezolid resistance was 0.4% (n = 5) in *S. capitis*, 0.4% (n = 17) in *S. hominis*, 0.2% (n = 5) in *S. haemolyticus*, 0.1% (n = 5) in *S. epidermidis*, and absent in *S. warneri*. Mupirocin resistance was 14.8% in *S. epidermidis* (n = 987, of

note: 166 additional isolates tested as "I") and between 1.7% and 6.5% in other species.

#### 7.4.4.6 Other relevant species

Resistance in *S. lugdunensis* (n = 82, sixth most occurrent species) remained generally low: 11.9% (n = 5) to amoxicillin/clavulanic acid, 7.3% (n = 6) to oxacillin, 4.8% (n = 4) to ciprofloxacin, 15.4% (n = 10) to tetracycline, 3.7% (n = 3) to teicoplanin, and no resistance was observed to rifampicin, linezolid, and vancomycin.

*S. saprophyticus* (n = 45, seventh-most occurrent species) showed no resistance to ciprofloxacin, teicoplanin, rifampicin, and vancomycin. Resistance to erythromycin was 15.4% (n = 6), to linezolid 7.9% (n = 3), and to oxacillin 16.2% (n = 6).

*S. pettenkoferi* (n = 44, eighth-most occurrent species) showed no resistance to gentamicin, tobramycin, linezolid, teicoplanin, or vancomycin but resistance to oxacillin was 40.4% (n = 14). Resistance to ciprofloxacin (8.1%, n = 3) and trimethoprim/sulfamethoxazole (2.7%, n = 1) remained low.

#### 7.4.4.7 Effect of patient age groups on antibiotic resistance in CoNS

Thirty bug-drug combinations were analysed of which 13 showed a significant linear trend associated with age groups (Figure 4). In *S. epidermidis*, resistance to beta-lactam antibiotics was found to be lower in older patients (amoxicillin/clavulanic acid:  $p = 0.002$ ; cefuroxime:  $p = 0.014$ ). This was also observed in all aminoglycosides (e.g., gentamicin:  $p = 0.017$ ; tobramycin:  $p = 0.009$ ), except for kanamycin where higher age was associated with increasing resistance ( $p = 0.011$ ). *S. epidermidis* was also less resistant to carbapenems in older patients (imipenem:  $p = 0.046$ ; meropenem:  $p = 0.047$ ). In *S. hominis*, similar trends were observed, although the effect of resistance to kanamycin was stronger ( $p = 0.006$ ). *S. capititis* showed significantly more resistance to tetracycline ( $p = 0.022$ ) in older patients.

## 7.5 Discussion

The present study provides a comprehensive analysis of species in the CoNS group and their associated ABR patterns in a full-region approach using solely MALDI-TOF MS for discriminating CoNS species. We selected and analysed a total of 14,992 first weighted CoNS isolates from 10,786 patients over seven years and identified significant differences in the trends of occurrence of the different CoNS species as well as in their ABR patterns.

Before MALDI-TOF MS, CoNS were often reported without the species name as formerly used techniques were not able to reliably discriminate species<sup>[16]</sup>. The ratio of all CoNS species presented in the current study (Table 2) shows that five

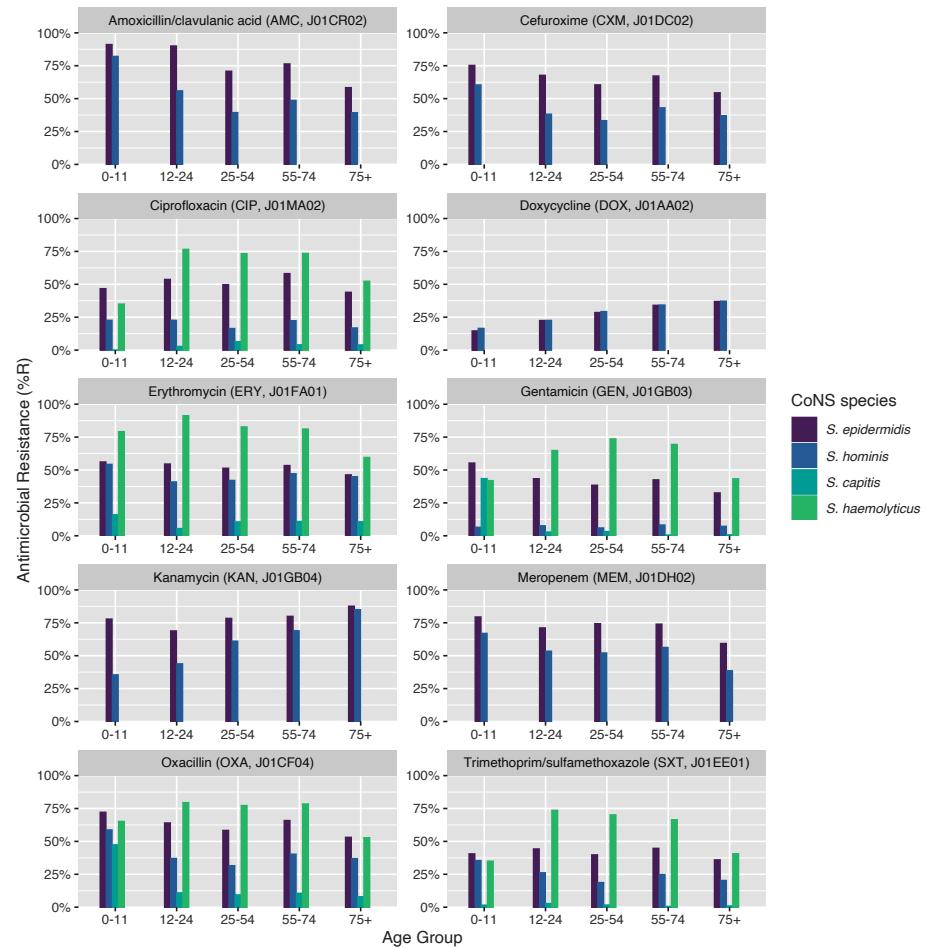


Figure 7.4: Age group comparison of ABR per antibiotic. Only bug-drug combinations are shown where at least 30 isolates were available for each age group and where results for all age groups were available.

species accounted for 97.1% of all 27 found CoNS species with *S. epidermidis* accounting for the largest subgroup (48.4%, n = 7,260). This distribution of species largely confirms results by previous reports [9,29].

For most CoNS species, pathogenicity has not been studied widely due to the lack of data. For this reason, we defined CoNS persistence as at least three positive blood cultures drawn on three different days within 60 days, containing the same CoNS species. This definition was applied for two reasons. Firstly, it rules out contamination since the chance of finding the same contaminating species three times on three different days is expected to be low. Secondly, it prevents underestimating the possible pathogenicity of CoNS species since three sequential findings indicate CoNS persistence. In total, 294 different cases of CoNS persistence were identified (Table 3) among the 10,786 included patients. *S. haemolyticus* was found to be proportionally more associated with CoNS persistence (5.8%) than *S. epidermidis* (3.7%) and *S. hominis* (0.9%), although the latter two were eight to ten times more prevalent than *S. haemolyticus*. *S. epidermidis* has widely been recognised as a pathogen and an important cause of BSIs [5,30]. It was probably found more often than *S. haemolyticus* due to its stronger association with skin colonisation [8] although we could not confirm this finding. It has been reported that *S. haemolyticus* is an emerging threat and one of the most frequent aetiological factors of staphylococcal infections [9,31]. Adding to this worrisome trend is the great concern of ABR in *S. haemolyticus* which was reported with 75% of analysed *S. haemolyticus* isolates to be multi-resistant [32]. We confirmed this in the present study in which the ABR analysis showed that 72.1% of *S. haemolyticus* isolates were resistant to oxacillin and 77.6% resistant to macrolides.

ABR analysis also showed substantial differences between CoNS species (Figure 3, Figure 4, Table 4). This observation could be supported by a recent study that showed strong heterogeneity in the resistance genes for CoNS species [33]. For example, the *blaZ* and *aac-aphD* genes that can lead to penicillin and aminoglycoside resistance, respectively, were found to be up to four times more common in *S. haemolyticus* than in other CoNS species [33]. The level of resistance to oxacillin and consequent amount of methicillin-resistant *S. epidermidis* (MRSE) identified in the present study (61.9%) could also be supported by the mentioned study, that reported high prevalence of *blaZ* in *S. epidermidis* (64.2%). Although differences in occurrence and ABR within CoNS species are known, they are often neglected, both in studies and in clinical practice. As an example, the Dutch national report on ABR and antibiotic consumption, NethMap, combines all CoNS species into one category making it impossible to distinguish between species. Nonetheless, Dutch treatment guidelines are based on NethMap [34]. As an example, in 2019 NethMap reported for isolates found on ICUs 0% linezolid resistance in CoNS, 8% rifampicin resistance, and more than 20% resistance in all other antibiotic classes in 2019. These results could be confirmed in the present study on the group level but not on the species level. The lack of acknowledging ABR differences within species might cause the development of treatment guidelines – and the subsequent future treatment

of BSI caused by CoNS – to focus on ‘ABR-safe’ agents for treating CoNS, such as vancomycin or linezolid. Still, agents such as tetracycline, co-trimoxazole, and erythromycin could be considered viable options for some species where, according to our results, ABR never surpassed 10%. Furthermore, as age showed to have a significant effect on ABR (Figure 4), treatment guidelines could also be improved by incorporating age-specific recommendations. We could not find the correlation between ABR in CoNS species and age in previous literature.

In the present study, some CoNS species are noteworthy to be highlighted. For instance, *S. pettenkoferi* was found only two to three times per year between 2013 and 2017 while this increased to 13 and 22 times per year in 2018 and 2019, respectively. Although recently named, multiple case studies showed that *S. pettenkoferi* was found to be the causative agent of septic shock, bacteraemia, and wound infections and has also shown resistance to linezolid [35–37]. Opposingly, no linezolid resistance was found in the present study. Cases of BSI caused by *S. pettenkoferi* could incorrectly be assigned to *S. capitis* that greatly resembles *S. pettenkoferi* [38]. The emerging neonatal pathogen *S. capitis* is another noteworthy species causing sepsis and manifesting as a multidrug-resistant microorganism [39]. In this study, 7.53% of all first weighted *S. capitis* isolates was found in one-year old children. Clinically relevant ABR (e.g., to chloramphenicol or vancomycin) was not found in these children in this study. This implies that the internationally emerging *S. capitis* NRCS-A clone [39] has not been found in the Northern Netherlands between 2013 and 2019.

Our study has limitations, mostly due to its sole source of routine diagnostic data. Firstly, it was not known which isolates were causal to BSI. This hinders the assessment of contamination as well as the determination of clinical importance. Secondly, the VITEK 2 systems between laboratories used different cartridges with different antibiotics which could lead to an incorporation bias towards some laboratories or hospitals. Additionally, the MALDI-TOF MS systems of all laboratories keep their taxonomic reference data, which is proprietary, and the recency could not be assessed. Thirdly, no genotyping was available for any of the included isolates since genotyping was not considered common practice for routine diagnostic workflows at the time of the study. For this reason, no assessment could be made about a hospital-associated cluster of strains. Lastly, vancomycin resistance might have been underdiagnosed in this study since Vitek2 AST is not optimal for testing glycopeptide resistance [40].

For the first time, a multi-year, full-region approach to comprehensively assess both the occurrence and ABR patterns of CoNS species based on MALDI-TOF MS results was carried out. Although CoNS often lack aggressive virulence properties, evaluating the occurrence and ABR patterns remains highly relevant [9]. Stratification by region and demography unveiled a large heterogeneity in ABR between species, settings, and age groups which could be used for (re-)evaluating treatment policies and understanding more about these important but still too often neglected pathogens.

## Supplementary tables

1. Supplementary Table 1 (file “supp\_tbl1.xlsx”): Extensive output of EU-CAST changes to the original data set.
2. Supplementary Table 2 (file “supp\_tbl2.xlsx”): List of species and all available AST test results. This file contains a SHA2 hash (256-bit) of the patient IDs, to be able to reproduce some part of the Results section on the patient level. The hash contains irretrievable information, rendering the data set strictly anonymous.
3. Supplementary Table 3 (file “supp\_tbl3.xlsx”): ABR analysis per species for all available variables.

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