

A New Instrument for Microbial Epidemiology

Empowering Antimicrobial Resistance Data Analysis

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Contents

Preamble	5
Colophon	7
Contents	9
1 General Introduction	11
1.1 Microbial epidemiology	11
1.2 Antimicrobial resistance in microorganisms	14
1.3 Data analysis using R	23
1.4 Setting for this thesis	24
1.5 Aim of this thesis and introduction to its chapters	26
References	27
2 Diagnostic Stewardship: Sense or Nonsense?!	33
Abstract	33
2.1 Introduction	34
2.2 The general concept	38
2.3 Conclusion	42
Financing	42
References	43
3 Introducing a New, Free, and Independent Method for Standardised, Reproducible and Reliable Analyses of Antimicrobial Resistance Data	45
Abstract	45
3.1 Background	46
3.2 Standardising AMR data analysis	48
3.3 Comparison with existing software methods	48
3.4 User feedback	50
3.5 Conclusion	50
References	51

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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Contents

Section I

1. General Introduction

2. Diagnostic Stewardship: Sense or Nonsense?!

Berends MS*, Luz CF*, Wouthuyzen-Bakker M, Märtsönen AG, Alffenaar JW, Dik JWH, Glasner C, Sinha BNM
Dutch Journal of Clinical Microbiology (2018) 26;3

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

Berends MS, Luz CF, Sinha BNM, Glasner C[‡], Friedrich AW[‡]
In preparation

Section II

4. AMR - An R Package for Working with Antimicrobial Resistance Data

Berends MS*, Luz CF*, Friedrich AW, Sinha BNM, Albers CJ, Glasner C
Journal of Statistical Software (2021), ahead of print

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

Luz CF, Berends MS, Dik JWH, Lokate M, Pulcini C, Glasner C, Sinha BNM
Journal of Medical Internet Research (2019) 21;6, e12843

6. Better Antimicrobial Resistance Data Analysis and Reporting in Less Time

Berends MS*, Luz CF*, Zhou X, Friedrich AW, Lokate ML, Sinha BNM[‡], Glasner C[‡]
medRxiv [preprint] (2021), 21257599

Section III

7. Trends in Occurrence and Phenotypic Resistance of Coagulase-Negative Staphylococci (CoNS) Found in Blood in the Northern Netherlands between 2013 and 2019
Berends MS, Luz CF, Ott A, Andriesse GI, Becker K, Glasner C‡, Friedrich AW‡
In preparation
8. Defining Multidrug Resistance of Gram-Negative Bacteria in the Dutch-German Border Region-Impact of National Guidelines
Köck R, Siemer P, Esser J, Kampmeier S, Berends MS, Glasner C, Arends JP, Becker K, Friedrich AW
Microorganisms (2018) 6;1
9. Changing Epidemiology of Methicillin-Resistant *Staphylococcus aureus* in 42 Hospitals in the Dutch-German Border Region, 2012 to 2016: Results of the Search-and-Follow-Policy
Jurke A, Daniels-Haardt I, Silvis W, Berends MS, Glasner C, Becker K, Köck R, Friedrich AW
Eurosurveillance (2019) 24;15
10. A Prospective Multicentre MDRO Screening Study on ICUs in the Dutch-German Cross-Border Region (2017-2018): The Importance of Healthcare Structures
Berends MS*, Glasner C*, Becker K, Esser J, Gieffers J, Jurke A, Kampinga G, Kampmeier S, Klont R, Köck R, Al Naemi N, Ott A, Ruis G, Saris K, Tami A, Van Zeijl J, Von Müller L, Voss A, Waar K, Friedrich AW
Eurosurveillance (2021), ahead of print

Section IV

11. Summary and Future Perspectives

Gearfetting yn Frysk

Samenvatting in het Nederlands

Zusammenfassung auf Deutsch

Alphabetical list of published work

Alphabetical list of related presentations

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Curriculum Vitae

* 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 ^[1]. The year 1854 was considered the worst year, when 23,000 people died in the United Kingdom, out of 16 million inhabitants (0.14%) ^[2]. 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 ^[3]. 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 ^[4]. Interestingly, the Italian Filippo Pacini managed to isolate the bacterium causing cholera, *Vibrio cholerae*, in 1854 – the same year that Snow investigated the outbreak ^[5]. Although it was not until 1884 that *V. cholerae* was formally given its name by the German Robert Koch ^[6].

In his book about the ‘cholera poison’, Snow famously wrote ^[4]:

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

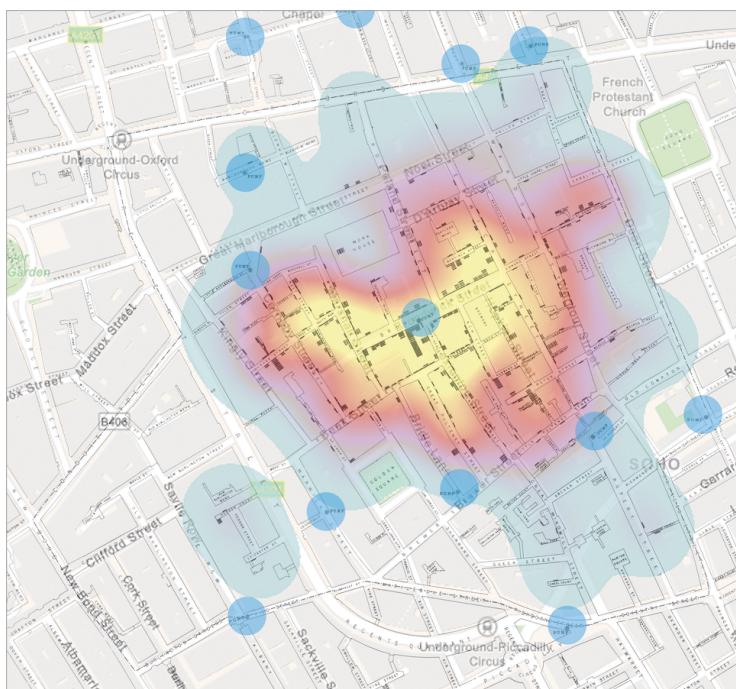


Figure 1.1: Visualisations of the ‘Broad Street cholera outbreak’ in London in 1854. Top: original map as drawn by John Snow. Bottom: Snow’s original map with a self-made heatmap visualisation overlay, based on the geographic position of the cases. The blue circles ($n = 13$) indicate the location of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

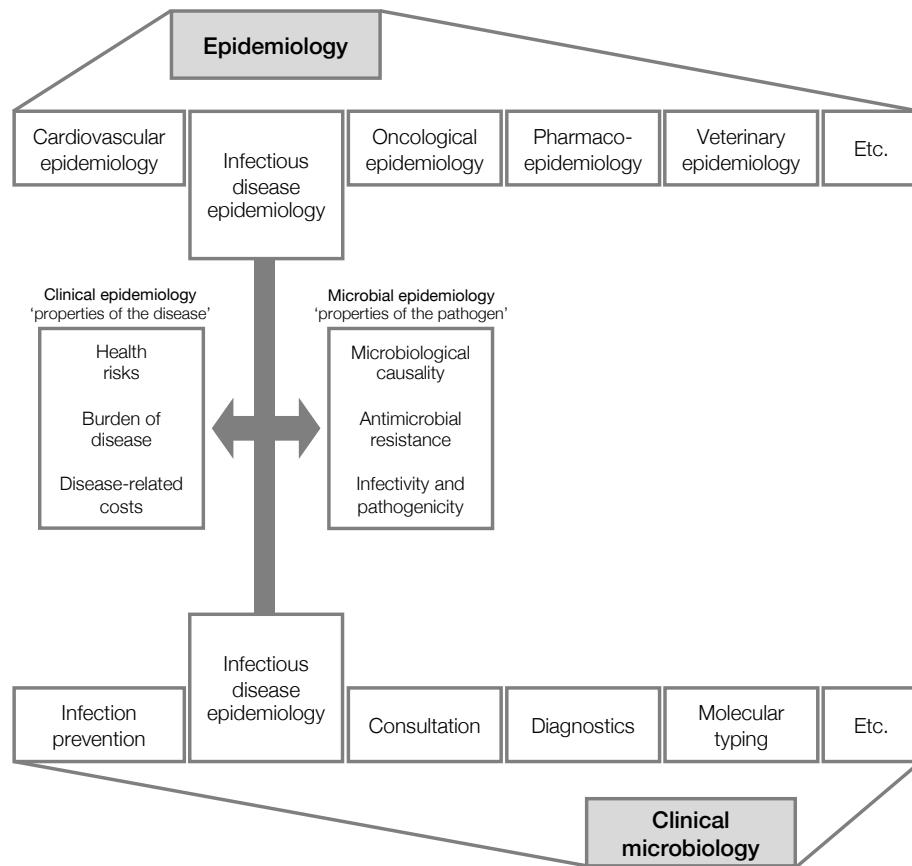


Figure 1.2: Overview of the diverse sections and subspecialties of epidemiology and clinical microbiology and their common field: infectious disease epidemiology. Microbial epidemiology can be considered to be a subspecialty of infectious disease epidemiology.

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 CO₂ 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 CO₂ 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

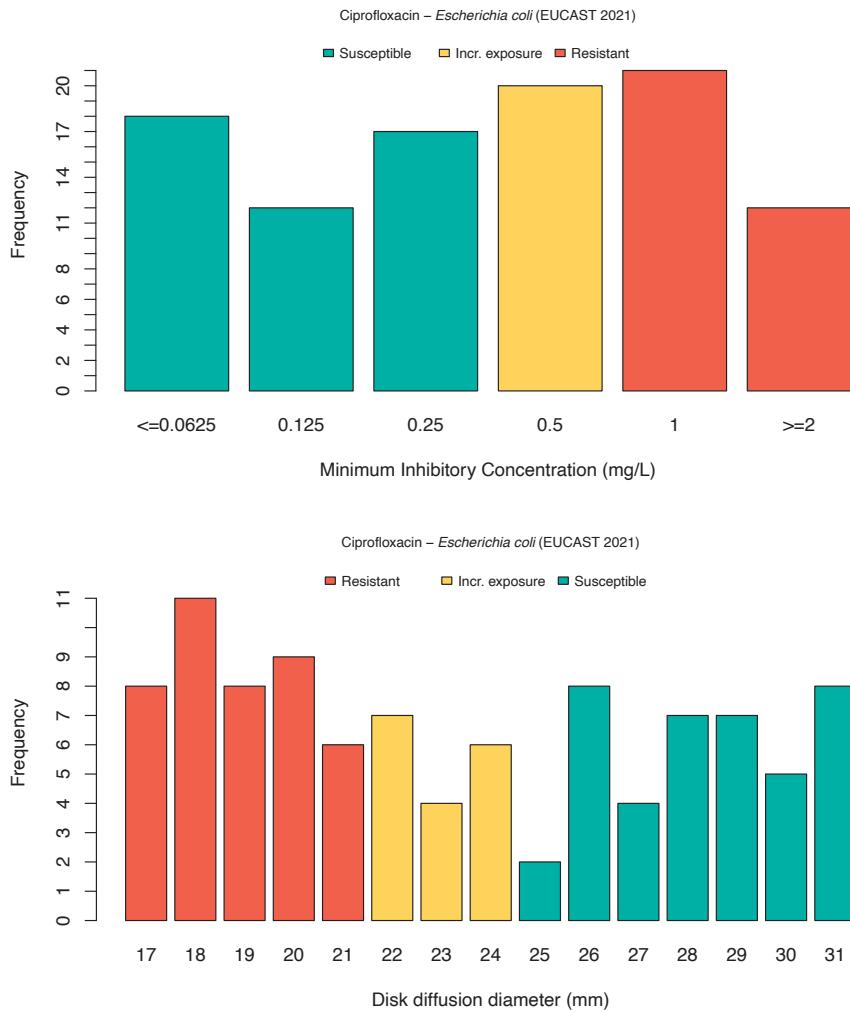


Figure 1.3: Interpretation of 100 random minimum inhibitory concentrations (top) and 100 random disk diffusion growth inhibition zones (bottom) of ciprofloxacin in **Escherichia coli**, interpreted using colours according to the EUCAST 2021 guideline. These plots were generated with the AMR package for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

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

national surveillance programs provide data for EARS-Net and GLASS of the 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

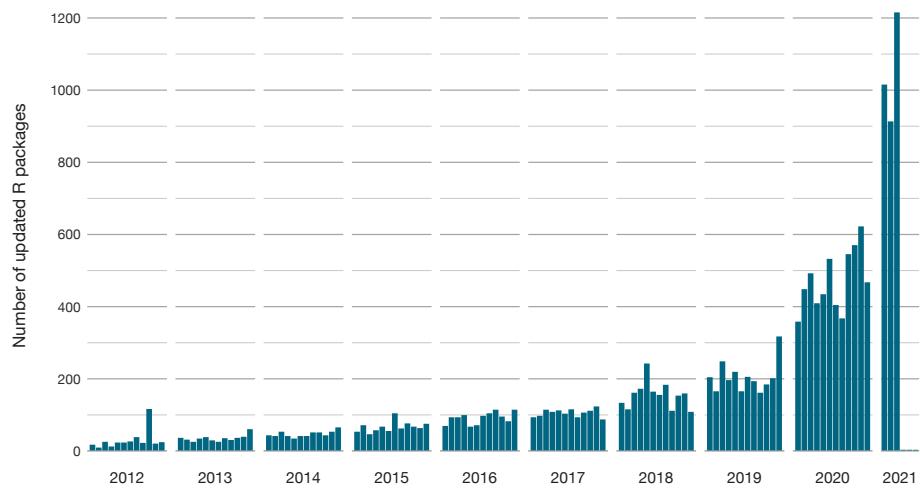


Figure 1.4: The number of R packages by date of the last update over the last ten years. Every bar represents one month. Every R package occurs once in this figure.

Consortium, that gathers experts in data science, modelling methodology, public 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 programming 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).

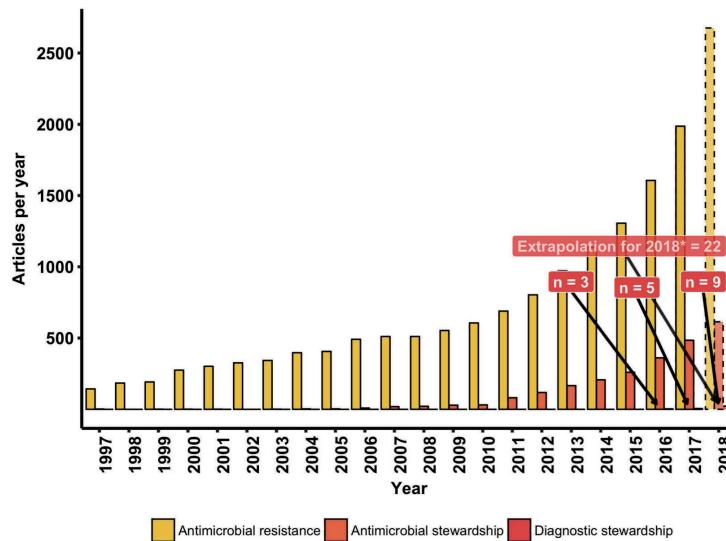


Figure 2.1: The increase of articles indexed in PubMed. Search strategies: 'antimicrobial stewardship'[Title/Abstract]; 'diagnostic stewardship'[Title/Abstract]; 'antimicrobial resistance'[Title/Abstract]. Source: <https://www.ncbi.nlm.nih.gov/pubmed/> (assessed: 2018-05-31).

* Extrapolation based on count from 2018-01-01 to 2018-05-31.

Nevertheless, the concept of DSP is neither intended to replace other stew-

ardship 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 loos-

36CHAPTER 2. DIAGNOSTIC STEWARDSHIP: SENSE OR NONSENSE?!

ening of the prosthesis - an indication for revision surgery. C-reactive protein (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.

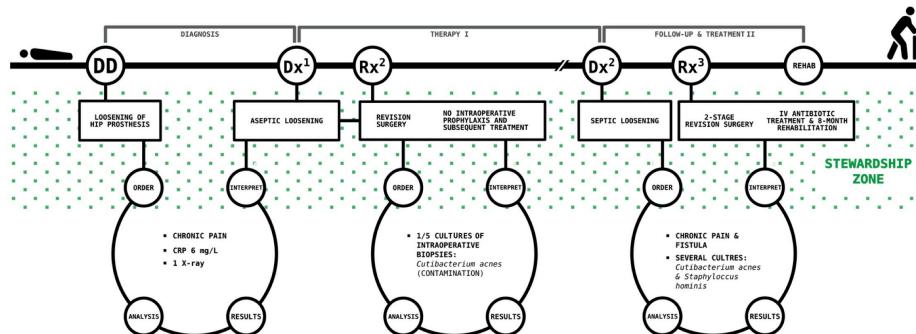


Figure 2.2: The first case.

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.

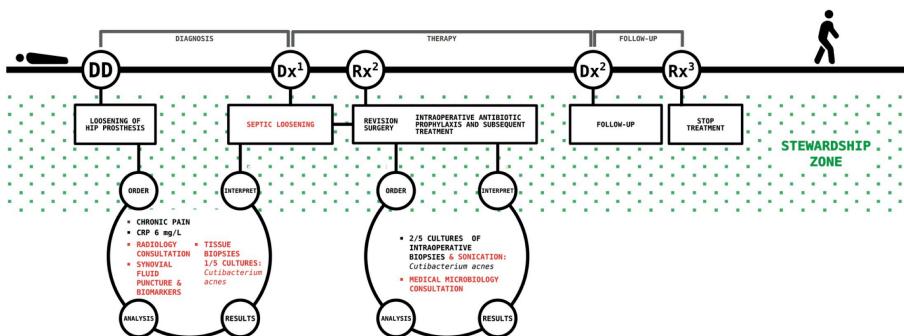


Figure 2.3: The second case.

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.

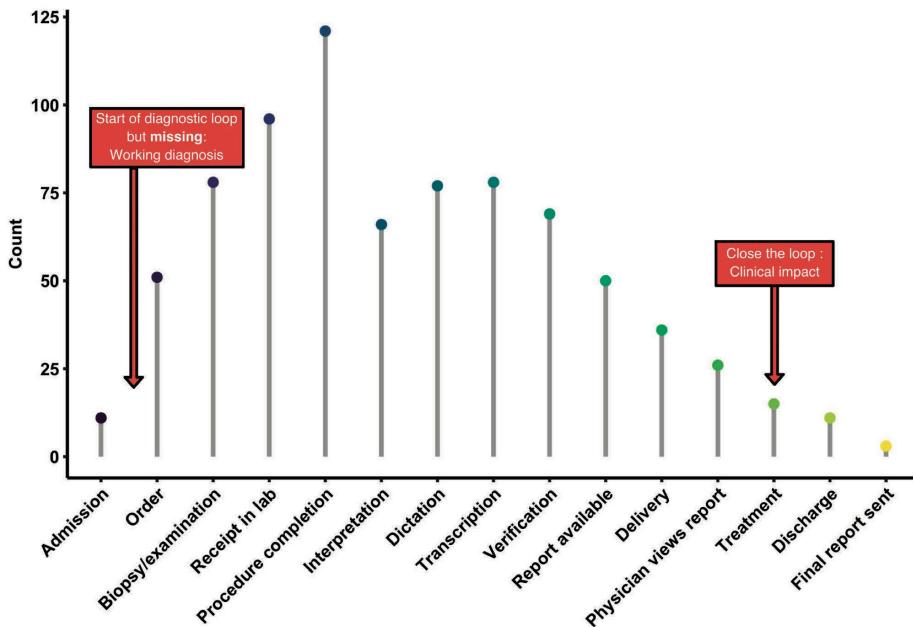
We embrace these developments but there are two aspects that are really essential for optimal quality of care. Both these aspects can be achieved through stewardship. Firstly, stewardship provides guidance for the appropriate choice of a customised diagnostic strategy for individual patients and patient 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.

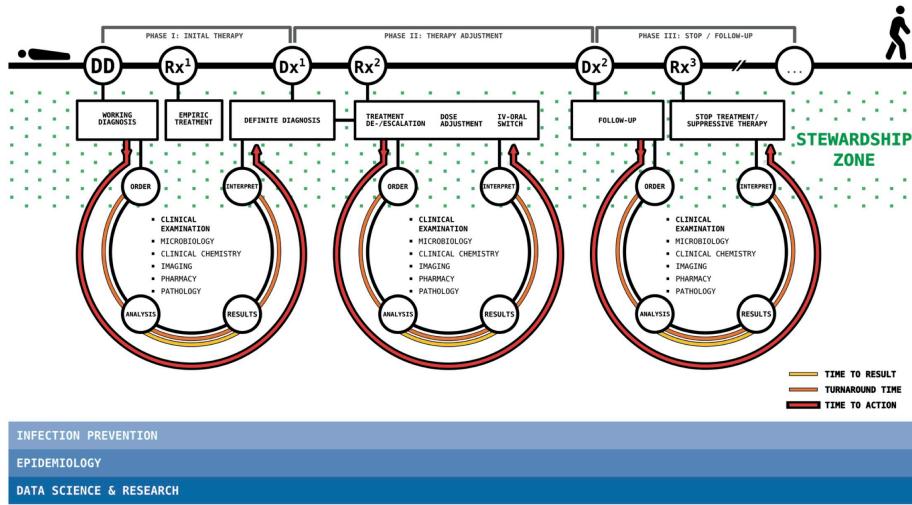


Figure 2.5: Stewardship in infection management.

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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44CHAPTER 2. DIAGNOSTIC STEWARDSHIP: SENSE OR NONSENSE??

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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

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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].

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



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.

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.

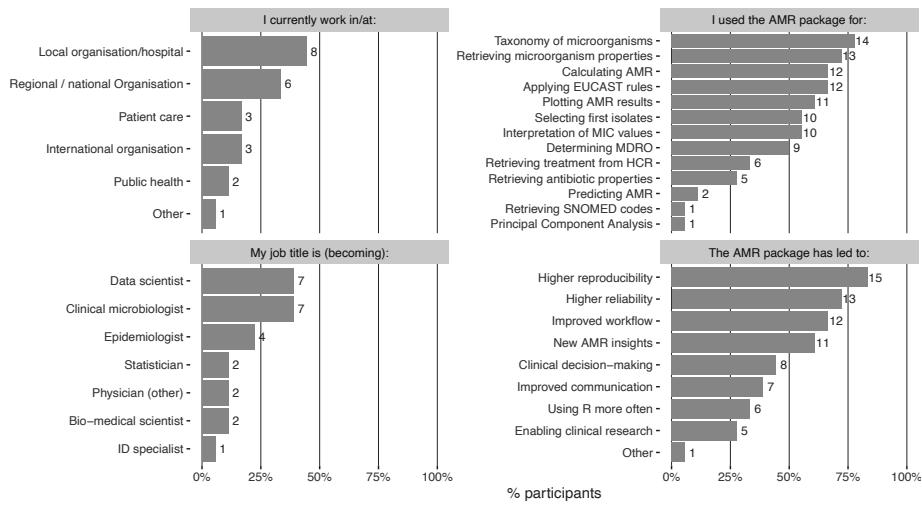


Figure 3.2: The outcome of the survey amongst 18 participants. MIC: minimal inhibitory concentration, MDRO: multidrug-resistant organism, SNOMED: Systematised Nomenclature of Medicine.

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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