Antibiotic resistance in specific sociodemographic groups

Implications for public health

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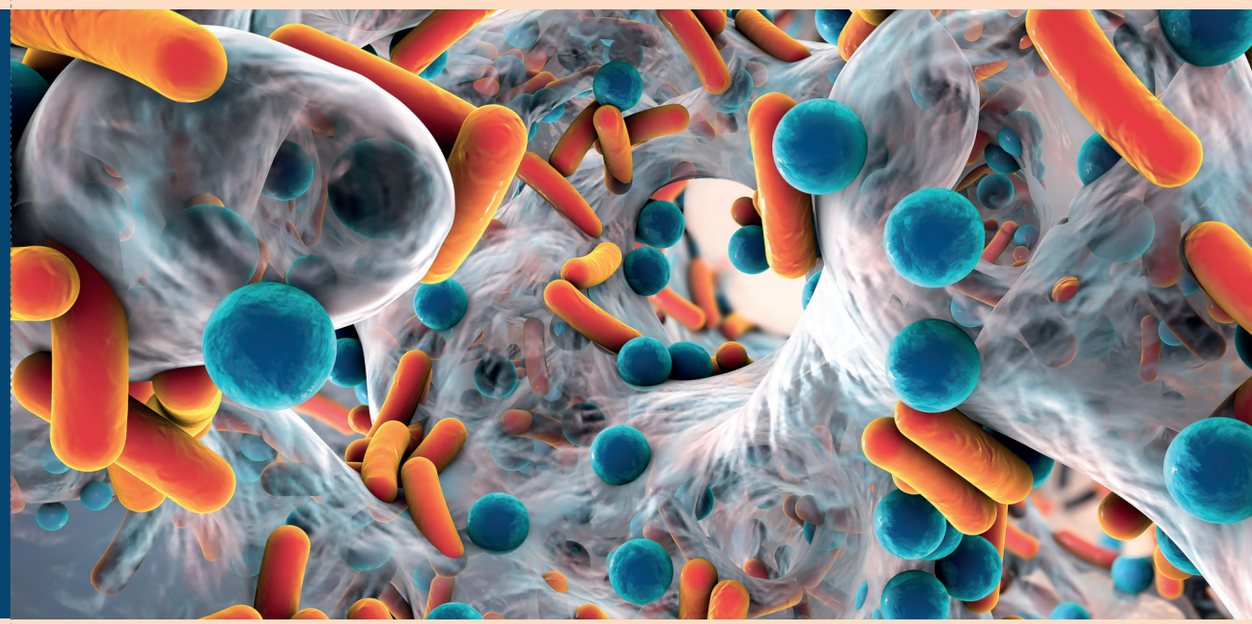

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Antibiotic resistance in specific sociodemographic groups: implications for public health

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General introduction

1.1 THE 'ANTIBIOTIC ERA'

During the 'pre-antibiotic era', bacterial, viral and parasitic infectious diseases were among the leading causes of human morbidity and mortality(1). Paul Ehrlich's magic bullet theory (i.e. targeting specific disease-causing microbes and not the host when treating infectious diseases) and Alexander Fleming's discovery of penicillin proclaimed the beginning of the modern 'antibiotic era'(2, 3). In 1942, penicillin was presented as the first clinically and commercially available β-lactam antibiotic, mainly to treat respiratory and skin infections, syphilis and meningitis(4). Improved hygiene (due to the construction of sewage systems and the availability of clean drinking water), vaccination against certain bacterial infections, such as diphtheria and whooping cough, and antibiotic treatment led to a drastic decline in mortality caused by bacterial infections(1, 5, 6). Over the past decades, many classes of antibiotics have become widely available and antibiotic treatment has saved millions of lives across the world.

1.2 THE EMERGENCE OF ANTIBIOTIC RESISTANCE

Alexander Fleming was one of the first warning that potential resistance of bacteria to penicillin could occur if treatment consisted of an insufficient dose or inadequate duration(3). Indeed, shortly after the introduction of a new antibiotic, resistance to the antibiotic was usually observed(7). Antibiotic resistance (ABR) occurs naturally, but (mis)use of antibiotics in humans and animals can accelerate the process(8). The three most important mechanisms of antibiotic resistance are enzymatic degradation of antibiotics, the alteration of bacterial proteins targeted by antibiotics and changes in the bacterial membrane that influence the permeability of antibiotics(9). The World Health Organization (WHO) warns that antibiotic resistance is increasing worldwide and that our ability to treat common infections such as urinary tract and skin infections is being threatened(10). Antibiotic (over)use in a community, inappropriate prescribing, extensive agricultural use and the availability of few new antibiotics are the main drivers of the current global increase in antibiotic resistance(11).

1.2.1 The burden of antibiotic-resistant infections

ABR leads to higher medical costs, extended hospital stays and increased mortality(8). In 2016, the final report of the Review on Antimicrobial Resistance suggested that infections by resistant micro-organisms (including multidrug-resistant tuberculosis) cause 700,000 individuals to die annually(10). It is estimated that in 2015 approximately 33,110 deaths attributable to infections with antibiotic-resistant bacteria occurred in the European Union (EU)(12). A Dutch study published in 2020 estimated that antibiotic-resistant

Gram-negative bacteria caused 12% of gram-negative infections in hospitalized patients between 2013 and 2016, although ABR did not increase 30-day mortality in Gram-negative infections(13). In 2019, a report from the Interagency Coordination Group on Antimicrobial Resistance (IACG) warned that, if no action is taken, the number of deaths could increase to 10 million annually around the world by 2050. In this scenario, 2.4 million people could die in high-income countries between 2015 and 2050(14).

Currently, the costs of ABR are not particularly high and do not significantly influence the world economy(15). However, the future costs of ABR are estimated to be potentially very high, amounting towards 100 trillion USD if no global action on ABR is taken(10, 15).

1.2.2 Drivers of antibiotic resistance

Use and overuse

ABR occurs naturally, but use and overuse of antibiotics in humans accelerate the evolution of resistance(8, 11), as an unavoidable consequence of the selective pressure of antibiotic use (16). Overuse of antibiotics mainly occurs by overprescribing, for instance when antibiotics are used to treat infections caused by other organisms than bacteria, which is a problem for many primary care settings(17, 18). Studies from the UK and Denmark indicate that approximately 80-90% of all antibiotic prescriptions are issued by general practitioners to treat respiratory tract infections(17, 19). Even though antibiotic use in primary care is relatively low in the Netherlands, a Dutch study published in 2014 showed that most antibiotics prescribed in primary care settings for upper respiratory tract infections and sinusitis were not in agreement with guideline recommendations. Inappropriate prescribing of antibiotics can also promote resistance in bacteria(11). Antibiotics are inappropriately prescribed when the incorrect type of antibiotic is selected, when suboptimal doses are prescribed or when the duration of treatment is too long. For example, suboptimal doses of antibiotics can promote selection of resistant bacterial subpopulations(20).

Insufficient knowledge about antibiotics and ABR, fear of complications and the tendency to be compliant with patients' wishes can lead to inappropriate prescribing of antibiotics(21, 22). Studies from the United States and France have shown that the indication for antibiotic treatment, choice of antibiotic and prescribed duration of treatment can be incorrect in 30% to 50% of antibiotic prescriptions(23, 24). Lack of public knowledge and awareness concerning antibiotics and resistance might also result in overuse of antibiotics, since expectations and knowledge of patients can potentially influence a physician's decision to prescribe antibiotics(22).

Overuse of antibiotics also occurs when antibiotics are prescribed as prophylaxis (for instance after surgery)(25, 26). When patients do not adhere to their antibiotic therapy or when the quantity of prescribed antibiotics exceeds the length of the treatment, antibiotics can be leftover(27). Subsequently, antibiotic misuse can occur when patients self-medicate with such leftover antibiotics from previous prescriptions(22, 27). Finally, in many countries outside the EU antibiotics can be bought 'over the counter' without a prescription, and the internet has made it possible to purchase antibiotics online, also in countries where the use of antibiotics is regulated (22, 28).

Extensive agricultural use

Antibiotics are extensively used in agriculture around the world to treat infections in livestock, which contributes to the development of resistance to antibiotics commonly used to treat human infections(8, 29). When animals are treated with antibiotics, this also selects for the development of antibiotic resistance. These resistant bacteria can subsequently be transmitted from animals to humans through direct contact, meat consumption, or contaminated water and soil(30). Additionally, antibiotics are used for the prevention of diseases and promotion of growth in farm animals(31, 32). The use of antibiotics as growth promoters has however been banned in the EU since 2006(33).

1.3 ANTIBIOTIC RESISTANCE IN THE NETHERLANDS

The Netherlands has one of the lowest prevalence of resistance and the least antibiotic-resistant infections in Europe and globally(12, 34). A large European population-level modelling analysis estimated that in the Netherlands, with around 17 million inhabitants, approximately 5000 infections with antibiotic-resistant bacteria occurred in 2015, resulting in 206 ABR attributable deaths, among which 187 deaths were due to Gram-negative infections(12). In the Netherlands, around 3000 patients die from Gram-negative infections annually(35). However, a recent study suggests that attributable mortality due to antibiotic-resistant Gram-negative infections in the Netherlands is negligible(13). Additionally, in the Netherlands, the prevalence of resistance among most pathogens is stable or declining(36).

The relatively low number of infections and ABR attributable deaths may attest to the success of the Dutch approach to tackle ABR. First, general physicians in the Netherlands are generally reluctant in prescribing antibiotics. In outpatient settings, total systemic use of antibiotics was 8.68 Defined Daily Dose (DDD)/1,000 inhabitant days in 2019, which is the lowest in the EU(36, 37). Additionally, professional treatment guidelines exist for both inpatient and outpatient settings(38-41). Furthermore, the Dutch search

and destroy policy for methicillin-resistant *Staphylococcus aureus* (MRSA) vigorously combats MRSA within hospitals(42). As part of this policy, all known MRSA carriers, as well as their household and in-hospital contacts, all patients who have been admitted to foreign hospitals for more than 24 hours in the previous 2 months, and patients who have been in contact with live pigs, calves and broilers are isolated at hospital admission until MRSA screening cultures are negative or carriage is eradicated(42, 43). Whereas 10 years ago antibiotic use in the Dutch veterinary sector was one of the highest in Europe(44), antibiotic use in agricultural and livestock settings has been drastically reduced with roughly 70% between 2009 and 2019 and sales of antimicrobial veterinary products decreased from 179 tons in 2018 to 150 tons in 2019(45, 46). The presence of ESBL producing *Escherichia coli* was lower in 2019 compared to 2018 among all livestock species(46). Lastly, since 2012, the so called 'A-teams' (antimicrobial stewardship teams) in hospitals improve the quality of hospital antibiotic use in the Netherlands.

1.3.1 National approach for antibiotic resistance

The most important goal of the national approach for ABR is to prevent the development and spread of resistance and to decrease resistant bacteria-related disease burden (morbidity and death)(47). By doing so, antibiotics will remain a valuable treatment option in the future. Since 2017, healthcare professionals from different care and cure facilities and organizations collaborate with public health professionals through ten regional care networks for ABR(48). Resistant bacteria can spread within cure and care institutions through patients and healthcare professionals, but can also be transmitted outside these settings. For instance, when a patient returns home after hospital admission, when a cure or care professional comes home from work or when patients are admitted to a long-term care facility. In the Netherlands, cure and care facilities are expected to battle ABR within the institution, but the responsibility is less clear when patients are transferred between institutions or return home. Therefore, the regional approach to battle ABR throughout the chain from community to cure and care is a crucial part of the national efforts in reducing ABR. The most important tasks of the regional care networks ABR include promoting collaboration between all healthcare partners in the region, improving participation in national surveillance, drafting regional risk profiles for the spread of ABR, improving the availability of and transparency concerning data on ABR, improving knowledge, skills and awareness concerning infection prevention and ABR among healthcare professionals and advising on ABR control measures(48).

1.4 MULTIDRUG-RESISTANT MICRO-ORGANISMS

Many clinically important multidrug-resistant micro-organisms (MDRO) have been established throughout the past decades(49). MDRO are organisms with acquired non-susceptibility to at least one agent in three or more antimicrobial categories(50). Clinically important MDRO are methicillin-resistant *Staphylococcus aureus* (MRSA), extended spectrum β-lactamase producing *Enterobacterales* (ESBL-E) and carbapenemase-producing gram-negative bacteria (CPB). MRSA and ESBL-E are among the most widespread MDRO in clinical and community settings globally(12, 51-53). CPB are abundant in some parts of the world, but are still relatively rare in Western and Northern European countries and in the USA (12, 54-58). However, infections with CPB are worrisome as they leave few therapeutic options(59, 60).

1.4.1 Methicillin-resistant Staphylococcus aureus

Staphylococcus aureus is a commensal bacterium, but can cause community-acquired or nosocomial infections(61). Nasal carriage of *S. aureus* is associated with increased risk of infection(62, 63). MRSA was described in 1961, shortly after the introduction of methicillin. Methicillin resistance is mediated by a modified penicillin-binding protein, PBP-2a, encoded by the *mecA* gene. This modified protein has a low affinity for β-lactams, allowing cell-wall biosynthesis to continue(64). In the early 1960s, outbreaks of MRSA were described among hospitalized patients in the UK and Denmark(65-67). When an infection with MRSA occurs, the remaining treatment options are rather limited. The drug of choice to treat most MRSA infections is vancomycin. Several other antibiotics such as daptomycin, telavancin, ceftaroline and linezolid can also be used to treat (invasive) MRSA(68).

Globally, most countries have a prevalence of MRSA carriage of more than 25%, some of which are higher than 50%(69, 70). Annually, approximately 7,000 MRSA attributable deaths occur in the European Union(12). The Dutch MRSA search and destroy policy, together with the general reluctance of Dutch general physicians to prescribe antibiotics, has resulted in a MRSA prevalence of <0.2% among hospital admissions and <1% among the general Dutch population(42, 71, 72). Increased risk of MRSA carriage has been described for several groups, including healthcare workers, adopted children from abroad living in the Netherlands, individuals who have been in contact with live pigs, veal calves or broiler chickens (LA-MRSA, or livestock-associated MRSA), and asylum seekers(43, 73-75).

1.4.2 Extended spectrum β-lactamase producing Enterobacterales

Bacteria that produce extended spectrum β-lactamases (ESBL) are able to break down commonly used β-lactam antibiotics(76). ESBL was acknowledged as clinically important among patients in France in 1984 and in the United States in 1988 and have become widespread ever since(77). The delay of adequate antibiotic treatment can lead to increased mortality in patients with ESBL-E infections(78, 79). ESBL-E is mostly transmitted through fecal-oral contact(80), but recently the possibility of sexual transmission of ESBL-E has also been suggested(81). Since 2000, ESBL producing Enterobacterales (ESBL-E) are increasingly found in both community-acquired infections and nosocomial infections(82-86). (87). Increased risk of ESBL-E carriage has been described for several groups, including patients who were previously colonized with ESBL-E (within one year), who received antibiotic treatment in the previous 30 days, farmers and travelers to high ESBL-E endemic regions(87-90).

A recent meta-analysis estimated the global pooled prevalence of intestinal carriage of ESBL *E. Coli* to be 16.5%. The highest carriage rates were observed in South-East Asia (27%) and the lowest carriage rates in Europe (6.0%)(91). The prevalence of ESBL-E is reported to be 5.0% among the general Dutch population(92) and to be 8.6% among the general population of Amsterdam, the Netherlands.

1.4.3 Carbapenemase-producing gram-negative bacteria

Carbapenems are last-resort β-lactam antibiotics, which are effective in severe infections caused by ESBL-E and other bacteria(93). Bacteria that produce the enzyme carbapenemase are able to break down almost all β-lactam antibiotics, including carbapenems(94). Carbapenem resistance was first reported in carbapenemase-producing *Enterobacteriales* in the early 1990s and has become a worldwide problem since(95). Carbapenemase-producing bacteria (CPB) such as carbapenemase-producing *Enterobacterales* (CPE), but also carbapenem-resistant *Acinetobacter spp.* (CRA) and carbapenem-resistant *Pseudomonas aeruginosa* (CRP) are categorized as priority-1 bacteria by the WHO, because they pose the biggest threat to human health(96). These bacteria can cause detrimental and often fatal infections such as bloodstream infections and pneumonia(96). Options for the treatment of carbapenem-resistant infections are very limited, with polymyxins, tigecycline, fosfomycin and aminoglycosides as drugs of choice(60). Efficacy of these drugs is usually limited and toxicity in general a problem. The most important risk factors for CPB acquisition during hospitalization are the use of medical devices and carbapenem use(97).

CPB are prevalent worldwide; however, differences in prevalence exist between and within continents and countries(54). It should be noted that sound estimations on the

prevalence of CPB are scarce due to incomplete reporting, changing definitions of the multidrug-resistant phenotype and diagnostic differences(54). Together, CPE, CRA and CRP cause almost 9.000 deaths in the European Union annually(12). CPB are rare In the Netherlands, but they are occasionally diagnosed in patients, mainly following hospital admission abroad(98). Based on data submitted by 28 Dutch laboratories to the Infectious diseases Surveillance System-Antibiotic Resistance (ISIS-AR), the overall prevalence of gradient test confirmed CPE has slightly increased in recent years, from 0.03% in 2015 to 0.08% in 2019 for *E. coli* and from 0.35 to 0.50% in *Klebsiella pneumoniae*(36).

1.5 AIMS AND OUTLINE OF THIS THESIS

This thesis aims to describe by means of epidemiological studies the occurrence of and associated factors with antibiotic resistance among specific sociodemographic groups living in Amsterdam, the Netherlands, and to elaborate on the implications for public health. This thesis focuses on ABR outside of hospital settings, since this is less extensively described in scientific literature compared to ABR in hospital settings and spread to other community members is likely. Consequently, preventative measures should be taken when a specific group is at serious increased risk.

In **part I** of this thesis, antibiotic knowledge, antibiotic use and prevalence of MRSA among different migrant groups living in Amsterdam, the Netherlands are studied. In **chapter 2**, knowledge and use of antibiotics among inhabitants of Amsterdam from six different ethnic groups who participated in the HELIUS study is described. **Chapter 3** describes whether measuring antibiotic knowledge differs by ethnic group. **Chapter 4** provides insight into the prevalence of nasal MRSA carriage among undocumented migrants and uninsured legal residents in Amsterdam.

Part II focuses on the prevalence of ESBL-E among specific groups in Amsterdam, the Netherlands. **Chapter 5** describes the carriage of ESBL-producing *Enterobacterales* and its association with sexual activity among men who have sex with men (MSM) participating in the Amsterdam Cohort Studies (ACS). In **chapter 6**, the prevalence of multidrug resistant *Enterobacterales* among residents of long-term care facilities in Amsterdam is described.

Part III, chapter 7 studies perceived barriers and enablers for preventing the spread of carbapenemase producing gram-negative bacteria during patient transfers between healthcare providers in the Dutch provinces of Noord-Holland and Flevoland.

Finally, **chapter 8** discusses the findings of this thesis and relates them to the most recent literature. Furthermore, recommendations for future research and prevention of ABR in public health settings are presented.

Table 1 provides an overview of data sources and study characteristics of studies included in this thesis.

Table 1. Data sources and study characteristics of studies included in this thesis

Data source/ recruitment site	Study design/type	Study population	Period of data collection	Chapter
HELIUS study	Cross-sectional	Random sample of Amsterdam residents of Surinamese, Turkish, Moroccan, Ghanaian and Dutch origin	2011-2015	2, 3
Kruispost*	Cross-sectional	Undocumented migrants and uninsured legal residents in Amsterdam	2018-2019	4
ABRACS	Cross-sectional	HIV-negative MSM	2018	5
Long term care facilities in Amsterdam	Cross-sectional	Residents of long- term care facilities in Amsterdam	2014-2015	6
Laboratories and care facilities in Amsterdam	Qualitative – quantitative (cross-sectional)	Healthcare providers of patients positive for carbapenem-producing *Enterobacterales*	2018-2019	7

* Kruispost is a Dutch primary health care facility (NGO)
Abbreviations: HELIUS, Healthy Life in an Urban Setting; ABRACS, Antibiotic Resistance in the Amsterdam Cohort Studies on hiv; HIV, human immunodeficiency virus; MSM, men who have sex with men; NGO, Non-governmental organization

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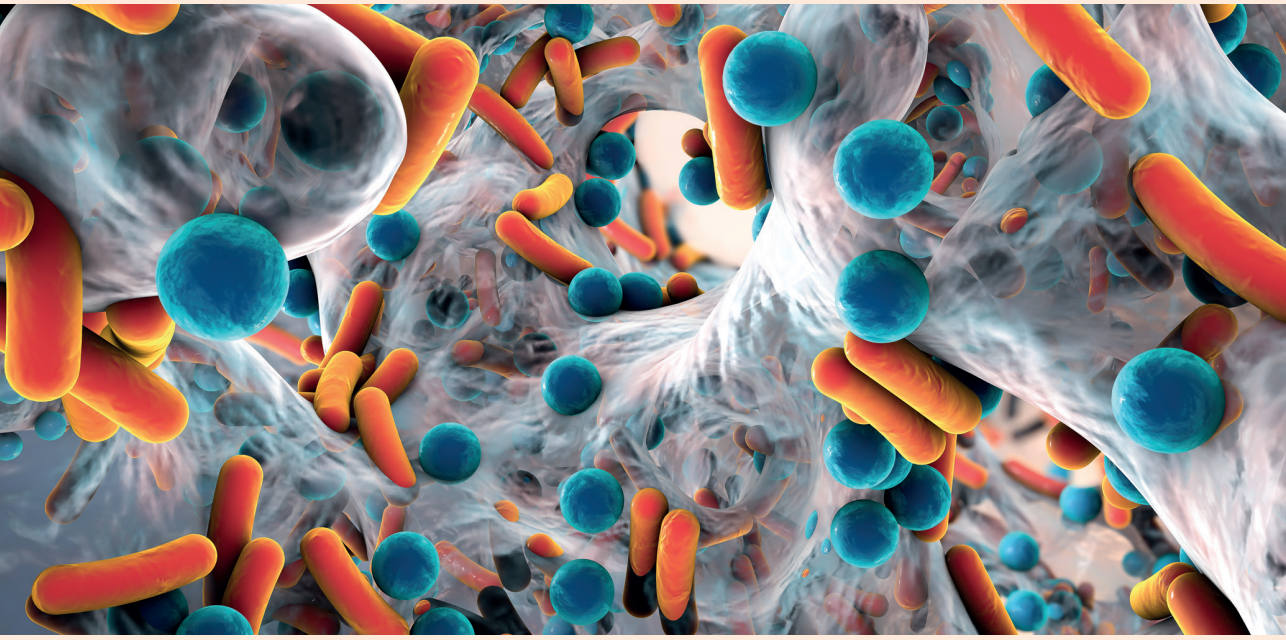

Part I

Antibiotic knowledge, antibiotic use and prevalence of methicillin resistant *Staphylococcus aureus* (MRSA) among different migrant groups living in Amsterdam, the Netherlands

2

Knowledge and use of antibiotics in six ethnic groups

The HELIUS study

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ABSTRACT

Background

The increase of antimicrobial resistance, mainly due to increased antibiotic use, is worrying. Preliminary evidence suggests that antibiotic use differs across ethnic groups in the Netherlands, with higher use in people of non-Dutch origin. We aimed to determine whether appropriate knowledge and use of antibiotics differ by ethnicity and whether knowledge on antibiotics is associated with antibiotic use.

Methods

We performed a cross-sectional study analyzing baseline data (2011-2015) from a population-based cohort (HELIUS study), which were linked to data from a health insurance register. We included 21,617 HELIUS participants of South-Asian Surinamese, African-Surinamese, Turkish, Moroccan, Ghanaian, and Dutch origin. 15,007 participants had available prescription data from the Achmea Health Data-base (AHD) in the year prior to their HELIUS study visit. Participants were asked five questions on antibiotic treatment during influenza-like illness, pneumonia, fever, sore throat and bronchitis, from which higher versus lower antibiotic knowledge level was determined. Number of antibiotic prescriptions in the year prior to the HELIUS study visit was used to determine antibiotic use.

Results

The percentage of individuals with a higher level of antibiotic knowledge was lower among all ethnic minority groups (range 57 to 70%) compared to Dutch (80%). After correcting for baseline characteristics, including medical conditions, first-generation African Surinamese and Turkish migrants received a significantly lower number of antibiotic prescriptions compared to individuals of Dutch origin. Only second-generation Ghanaian participants received more prescriptions compared to Dutch participants (aIRR 2.09, 95%CI 1.06 to 4.12). Higher level of antibiotic knowledge was not significantly associated with the number of prescriptions (IRR 0.92, 95%CI 0.85 to 1.00).

Conclusions

Levels of antibiotic knowledge varied between ethnic groups, but a lower level of antibiotic knowledge did not correspond with a higher number of antibiotic prescriptions.

BACKGROUND

The emergence of antimicrobial resistance, along with the steady decline in antibiotic development, has been identified as a major health threat for the coming decade by the World Health Organization (WHO). Increase in antibiotic use is the main reason for this development(1) and as such, antibiotics should only be prescribed when there is a clear indication for use.

A recent meta-analysis showed a higher prevalence of antimicrobial resistance among migrants in Europe.(2) There is preliminary evidence in the Netherlands that the use of antibiotics also differs across ethnic groups, with a higher use of antibiotics among people of non-Dutch origin.(3) The reason for this difference, however, is unclear. It could be explained by increased incidence of bacterial infections, but, to the best of our knowledge, there is no evidence to support this hypothesis. Alternatively, knowledge about antibiotic use might vary across ethnic groups. As expectations and knowledge of the patient could potentially drive a physician's decision to prescribe antibiotics, receiving prescriptions could also differ between ethnic groups.(4-6) There are also cultural-specific approaches to dealing with authority, being the physician in this setting, which have explained differences in antibiotic use between countries.(7)

The HELIUS (Healthy life in an Urban Setting) study is a large-scale, population-based cohort study among different ethnic groups, which was established with the aim to investigate mechanisms underlying the impact of ethnicity on communicable and non-communicable diseases.(10, 11) In 2018, approximately 13% of the population of the Netherlands was of non-Western origin. (8) The largest non-Western population groups were individuals of Turkish (2.4%), Moroccan (2.3%) and Surinamese (2.0%) descent. (8) In Amsterdam, approximately 36% of the population in 2018 was of non-Western descent. (9) The ethnic groups included in the HELIUS study are the largest ethnic minority groups of Amsterdam.(11) Amongst other data, data on antibiotic knowledge were collected. We were able to link these data at the individual level to data from a health insurance register on recent antibiotic use.

This study then provides a unique opportunity to determine whether knowledge about and use of antibiotics vary between ethnic groups, and if so, whether differences in antibiotic use can be attributed to differences in knowledge about antibiotics. We hypothesized that antibiotic use differs among ethnic groups as a result of differences in knowledge.

METHODS

Study population and design

The HEalthy LIfe in an Urban Setting (HELIUS) study is a multiethnic cohort study conducted in Amsterdam, which focuses on cardiovascular disease (e.g. diabetes), mental health (e.g. depressive disorders), and infectious diseases.(10, 11) In brief, baseline data collection took place in 2011–2015 and included people aged 18 to 70 years of Dutch, Surinamese, Ghanaian, Moroccan, and Turkish origin. A random sample of participants, stratified by ethnic origin, was taken from the municipality register of Amsterdam. Participants filled in an extensive self-administered questionnaire (variables included in the questionnaire are described elsewhere) (11) and underwent a physical examination during which biological samples were obtained.(11) No information was provided regarding appropriate antibiotic use. Between 2011 and 2015, 24,789 persons were included. Data collection procedures have been previously described in detail.(11) Both questionnaire data and physical examination data were available for 22,165 participants. The HELIUS study was conducted in accordance with the Declaration of Helsinki and was approved by the AMC Ethical Review Board. All participants provided written informed consent.

Ethnicity was defined according to the country of birth of the participant as well as that of their parents.(12) Specifically, a participant is considered to be of non-Dutch ethnic origin if they fulfill either of the following criteria: (1) they were born abroad and had at least one parent born abroad (first generation) or (2) they were born in Netherlands but both their parents were born abroad (second generation). -Dutch participants were born in the Netherlands and had both parents who were born in the Netherlands. After HELIUS data collection, the Surinamese group were further classified according to self-reported ethnic origin (obtained by questionnaire), into ‘African Surinamese’, ‘South-Asian Surinamese’, ‘Javanese Surinamese’ and ‘other/unknown Surinamese’.

Data linkage

Permission to link participants’ individual data to outside health registries was asked in the written informed consent form.(10) Of the 22,165 HELIUS participants, 19,895 agreed. HELIUS data of these individuals were linked to reimbursement data from the Achmea insurance company (Achmea Health Database, AHD) from 2010 until 2015. The AHD, obtained from the largest health insurance company in Amsterdam, contains all healthcare expenditures of every insured participant, including medications. A trusted third party linked data on reimbursed antibiotic prescriptions using an encrypted social security number and returned data without any identifying information. Procedures were in accordance with the General Data Protection Regulation.(13)

Inclusion and exclusion criteria for present study

Of the 22,165 participants, we excluded those of Javanese Surinamese or other/unknown Surinamese origin and those with another/unknown ethnic origin because of small participant numbers. For analyses on antibiotic use, we included those who gave permission for data linkage and could be linked to the AHD. To reduce bias for individuals with short-term insurance, we excluded those who were insured with Achmea for less than 365 days in the year preceding their HELIUS study visit.

Outcome variables

The primary outcomes were level of antibiotic knowledge and antibiotic use during the year prior to the HELIUS visit. Level of antibiotic knowledge was based on five questions, used in other studies (4, 6, 12), which asked the perceived necessity (yes/no) for antibiotic treatment during influenza-like illness, pneumonia, fever, sore throat and bronchitis.(4, 6, 14) Using these questions, we created an overall knowledge score of antibiotic use by summing the total number of correct responses, resulting in a score ranging from 0-5. A two-parameter logistic regression model was fitted to the five binary items based on the assumptions of item response theory (see Supplementary methods). From this model, “higher” and “lower” levels of antibiotic knowledge were defined by a knowledge score of ≥4 and <4, respectively.

Antibiotic use was obtained from linked AHD data and was based on the total number of reimbursed antibiotics (classified by ATC code J01; anti-infectives for systemic use) dispensed by community pharmacies from 2010 until 2015. We evaluated antibiotic use (yes/no) in the year prior to the HELIUS study visit, as well as the number of antibiotic prescriptions over the past year and during the entire insured period.

Other variables

Independent variables were obtained from the HELIUS study questionnaire (migration generation; sex; age; level of education; marital status; self-reported medical conditions; smoking; alcohol consumption; difficulty with the Dutch language and perceived health) and physical examination (body mass index (BMI, kg/m^2)). . Variables on antibiotic-related behavior were: not having finished antibiotic treatment; having saved antibiotics for later; and ever having asked the general practitioner (GP) for antibiotics. Definitions and grouping of variables are extensively described elsewhere.(10)

Statistical analyses

Sociodemographics, health status, antibiotic knowledge level and questions on antibiotic use were presented by ethnicity. To assess selection bias resulting from AHD data linkage, the same variables were compared between participants who were successfully

versus unsuccessfully linked. Comparisons between ethnic groups were made using a Pearson's χ^2 or Fisher exact test for categorical data and Kruskal-Wallis rank test for continuous variables.

Analysis on level of antibiotic knowledge included all HELIUS participants with available data. Odds ratios (OR) comparing levels of antibiotic knowledge across determinants and their 95% confidence intervals (CI) were estimated using logistic regression. All variables with an associated *p*-value <0.2 in univariable analyses were included in a full multivariable model and variables with a *p*-value above this level were removed in backwards-stepwise fashion. Given that the research aim was to determine differences between ethnicity, ethnic groups were forced in all models. This multivariable approach was chosen to not only assess other variables associated with antibiotic knowledge, but also to understand the extent of confounding bias when assessing the relationship between ethnicity and outcome variables.

Analysis on antibiotic use in the year prior to HELIUS study visit included all HELIUS participants who were linked to the AHD and were insured for at least 365 days with Achmea in the year prior to their HELIUS study visit. Determinants for having received ≥1 antibiotic prescription were assessed using logistic regression. The same multivariable approach as above was used for this outcome. We also compared antibiotic use during the entire period insured at Achmea versus the year prior to HELIUS study visit to assess differences when considering longer time periods.

Determinants for the total number of antibiotic prescriptions were then evaluated. As this outcome contained a high proportion of zero values and was over-dispersed, we used a zero-inflated negative binomial regression model. This model contains two parts: one accounting for zero values in the count distribution(zero-inflated) and another accounting for the over-dispersed count distribution (negative binomial). Covariates for the zero-inflated part were determined *a priori* from the risk-factor analysis on ≥1 antibiotic prescription. Covariates for the negative binomial part were selected from covariates with a *p*-value <0.2 in univariable analyses and variables above this *p*-value were removed in backwards-stepwise fashion. Incidence risk ratios (IRR) comparing the number of antibiotics prescribed over the past year across levels of determinants were estimated from this model.

Multicollinearity was verified using variance inflation factors, while any variable with an inflation factor of ≥4 was considered multicollinear and excluded from the model. To understand whether the association between ethnicity and outcome was modified by

demographic variables, interaction between ethnicity and other demographic variables was also assessed in all multivariable models.

The three variables involving antibiotic-related behavior were not initially considered in the final multivariable models. To assess whether ethnic differences in antibiotic use could be explained by patterns of antibiotic-related behavior, additional multivariable models including these variables were constructed for the endpoints (i) having received ≥1 antibiotic prescription and (ii)total number of antibiotic prescriptions.

Figure 1 provides an overview of all descriptive analysis and modeling used in the study. Significance was determined using a p-value <0.05. All analyses were conducted with Stata 13.1 (StataCorp., College Station, Texas, USA).

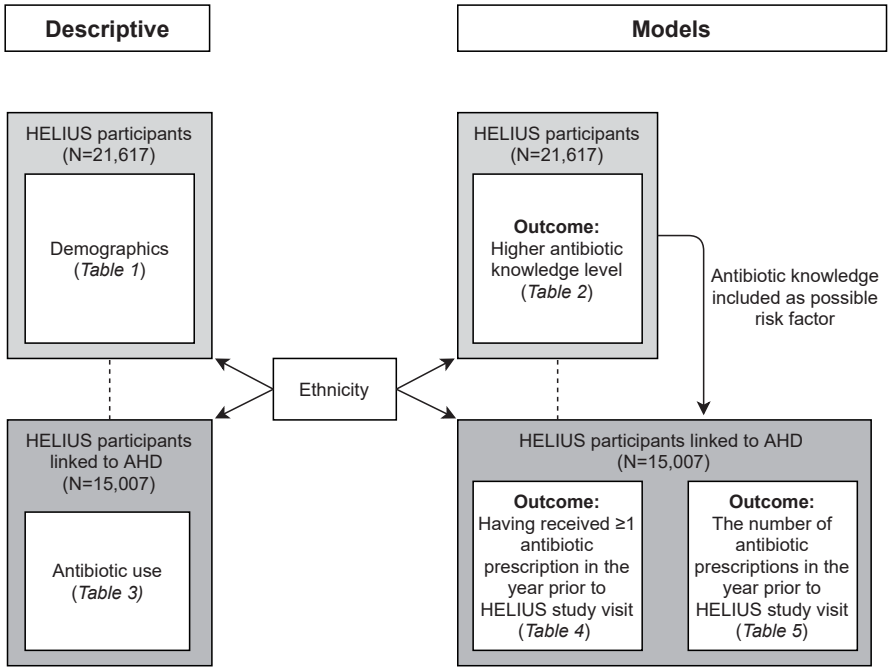

Figure 1. Overview of descriptive analysis and models used in the study.

RESULTS

Participants

Of the 22,165 HELIUS participants with available data, 21,617 were eligible after applying exclusion criteria. Their baseline characteristics, stratified by ethnicity, are shown in Table 1. Median age of participants was 46 years (IQR 34 to 55) and 58% were women. The proportion of several medical conditions predisposing individuals to antibiotic treatment differed by ethnicity. Of these conditions, South-Asian Surinamese participants had the highest prevalence of self-reported diabetes mellitus (17%) and cerebrovascular accident (CVA) (6%) over the last 12 months. Turkish individuals had more prevalent artery stenosis (10%), severe or chronic fatigue (45%) and respiratory diseases (15%), whereas Ghanaians more frequently reported high blood pressure (33%). Excellent perceived health was reported in 12% of Dutch participants in contrast to 3.3% of Turkish participants.

Ethnic differences in antibiotic knowledge

In several ethnic groups, there were substantial proportions of individuals reporting the need to be treated with antibiotics for illnesses without indication, as shown in Table 1. The number of people reporting to have been treated with antibiotics and not having regularly completed their antibiotic treatment was low across all ethnic groups, ranging from 0.1% in Dutch participants to 2.1% in Ghanaian participants. Few individuals regularly saved their antibiotics for later use, ranging from <0.1% in Dutch participants to 0.3% in Turkish participants. The percentage of participants having regularly asked their GP for antibiotics ranged from 0.6% in African Surinamese participants to 1.9% in Turkish and Moroccan participants.

As shown in Table 2, there was a significantly lower odds of individuals with higher level of antibiotic knowledge among all non-Dutch ethnic groups compared to Dutch individuals (overall $p<0.001$) (table 2). Across all non-Dutch groups, second-generation participants had a higher level of antibiotic knowledge than first-generation participants; however, results remained significantly lower compared to the Dutch group.

In multivariable analysis, all ethnic minority groups had lower odds for higher level of antibiotic knowledge compared to Dutch (overall $p<0.001$), although the effect for second-generation Ghanaian participants was not statistically significant. The odds for higher level of antibiotic knowledge were higher in all age groups >25 years of age (except for those ≥65) when compared to ≤25 years of age. Furthermore, women had a significantly higher odds of having a higher level of antibiotic knowledge compared to males. Lower odds for a higher level of antibiotic knowledge were found for the follow-

Table 1. Characteristics of the HELIUS study population (N=21,617) by ethnicity

Variables#	Ethnicity											
	Dutch (N=4,564)		South-Asian Surinamese (N=3,043)		African Surinamese (N=4,151)		Ghanaian (N=2,339)		Turkish (N=3,614)		Moroccan (N=3,906)	
Sociodemographics												
Female sex	2,475	54%	1,672	55%	2,535	61%	1,434	61%	1,980	55%	2,392	61%
Age in years, median (IQR)	47	(34-58)	48	(35-56)	50	(40-57)	47	(38-53)	42	(31-50)	40	(30-50)
Migration generation												
1st generation	N.A.	N.A.	2,328	77%	3,468	84%	2,231	95%	2,544	70%	2,680	69%
2nd generation	N.A.	N.A.	715	23%	683	16%	108	4.6%	1,080	30%	1,226	31%
Educational level												
Unknown	25	0.6%	16	0.5%	36	0.9%	42	1.8%	38	1.1%	38	1.0%
No school/elementary school	150	3.3%	437	14%	231	6%	660	28%	1,135	31%	1,205	31%
Lower vocational/lower secondary school	646	14%	1,010	33%	1,477	36%	917	39%	889	25%	694	18%
Intermediate vocational/ intermediate secondary school	994	22%	885	29%	1,464	35%	578	25%	1,020	28%	1,294	33%
Higher vocational/university	2,749	60%	695	23%	943	23%	142	6%	532	15%	675	17%
Marital status												
Married/registered partnership	1,724	38%	1,043	34%	766	19%	420	18%	2,208	61%	2,285	59%
Cohabiting	914	20%	311	10%	441	11%	427	19%	132	3.7%	110	2.8%
Unmarried/never married	1,474	32%	1,001	33%	2,231	54%	779	34%	761	21%	1,010	26%
Divorced/separated	356	8%	580	19%	617	15%	656	28%	407	11%	414	11%

Table 1. Characteristics of the HELIUS study population (N=21,617) by ethnicity *(continued)*

Variables#	Ethnicity											
	Dutch		South-Asian Surinamese		African Surinamese		Ghanaian		Turkish		Moroccan	
	(N=4,564)		(N=3,043)		(N=4,151)		(N=2,339)		(N=3,614)		(N=3,906)	
Widow/widower	87	1.9%	92	3.0%	65	1.6%	23	1.0%	90	2.5%	69	1.8%
Health status												
Self-reported medical conditions (previous 12 months)												
Diabetes mellitus	102	2.2%	521	17%	419	10%	185	8%	336	9%	389	10%
CVA/one-sided loss of bodily function ≤1 day	160	3.5%	212	7%	261	6%	95	4.1%	196	5%	195	5%
MI incl. ≥half hour chest pain or dotter/bypass operation	233	5%	491	16%	440	11%	225	10%	591	16%	476	12%
Severe heart condition	67	1.5%	120	4.0%	105	2.5%	75	3.2%	153	4.3%	58	1.5%
Malignant disorder	103	2.3%	70	2.3%	85	2.1%	33	1.4%	73	2.0%	46	1.2%
Severe or chronic fatigue	633	14%	1,032	34%	956	23%	186	8%	1,602	45%	1,465	38%
High blood pressure	534	12%	720	24%	1,230	30%	770	33%	610	17%	546	14%
Artery stenosis	85	1.9%	193	6%	181	4.4%	115	5%	348	10%	200	5%
Respiratory diseases	345	8%	433	14%	354	9%	117	5%	556	15%	446	11%
Serious/persistent intestinal disorders	249	5%	248	8%	308	7%	70	3.0%	433	12%	391	10%
Psoriasis	136	3.0%	168	6%	128	3.1%	71	3.1%	154	4.3%	121	3.1%
(Chronic) eczema	420	9%	406	13%	370	9%	71	3.1%	471	13%	423	11%
Incontinence	309	7%	326	11%	342	8%	108	4.7%	464	13%	300	8%

Table 1. Characteristics of the HELIUS study population (N=21,617) by ethnicity *(continued)*

Variables#	Ethnicity											
	Dutch (N=4,564)		South-Asian Surinamese (N=3,043)		African Surinamese (N=4,151)		Ghanaian (N=2,339)		Turkish (N=3,614)		Moroccan (N=3,906)	
Body Mass Index (kg/m^2), median (IQR)	24.1	(21.9-26.7)	25.7	(23.2-28.8)	27.0	(23.9-30.8)	27.9	(25.0-31.2)	27.9	(24.6-31.7)	27.0	(23.9-30.7)
Smoking												
Yes	1,129	25%	861	28%	1,309	32%	104	4.5%	1,240	35%	525	13%
No, never	1,689	37%	1,758	58%	2,016	49%	2,027	87%	1,700	47%	2,874	74%
No, but ever	1,737	38%	413	14%	805	19%	191	8%	648	18%	492	13%
Alcohol consumption												
Never	297	7%	1,072	35%	1,002	24%	806	35%	2,414	67%	3,265	84%
Not in previous 12 months	110	2.4%	251	8%	292	7%	408	18%	358	10%	338	9%
Monthly or less	436	10%	758	25%	1,242	30%	508	22%	367	10%	127	3.3%
2-4 times per month	894	20%	541	18%	873	21%	291	13%	257	7%	87	2.2%
2-3 times per week	1,413	31%	262	9%	439	11%	193	8%	132	3.7%	56	1.4%
≥4 times per week	1,408	31%	147	5%	272	7%	109	4.7%	57	1.6%	16	0.4%
Difficulty with Dutch language												
Yes	N.A.	N.A.	711	23%	520	13%	1,926	83%	2,136	60%	1,774	46%
Perceived health												
Excellent	541	12%	162	5%	303	7%	226	10%	117	3.3%	166	4.3%
Very good	1,381	30%	310	10%	571	14%	458	20%	383	11%	384	10%
Good	2,205	48%	1,623	53%	2,335	56%	1,180	51%	1,871	52%	1,871	48%

Table 1. Characteristics of the HELIUS study population (N=21,617) by ethnicity *(continued)*

Variables#	Ethnicity											
	Dutch		South-Asian Surinamese		African Surinamese		Ghanaian		Turkish		Moroccan	
	(N=4,564)		(N=3,043)		(N=4,151)		(N=2,339)		(N=3,614)		(N=3,906)	
Mediocre	402	9%	811	27%	834	20%	383	16%	921	26%	1,223	31%
Bad	28	0.6%	131	4.3%	101	2.4%	88	3.8%	307	9%	241	6%
Antibiotics												
Knowledge concerning antibiotics†												
Antibiotics effective for influenza	324	7%	554	19%	592	15%	658	29%	744	21%	648	18%
Antibiotics effective for pneumonia	4,166	92%	2,304	77%	3,114	77%	1,312	58%	2,587	73%	2,741	73%
Antibiotics effective for fever*	689	15%	552	19%	679	17%	586	26%	898	26%	614	17%
Antibiotics effective for sore throat*	672	15%	760	26%	1,089	27%	720	32%	1,203	34%	978	26%
Antibiotics effective for bronchitis	2,246	50%	1,385	50%	1,862	46%	919	41%	1,235	35%	1,485	40%
Higher level of antibiotic knowledge**	3,638	80%	1,996	68%	2,737	69%	1,248	57%	2,128	62%	2,528	70%
Did not finish antibiotic treatment												
Yes, regularly	5	0.1%	49	1.6%	44	1.1%	48	2.1%	41	1.2%	44	1.1%
Yes, occasionally	332	7%	312	10%	527	13%	174	8%	424	12%	445	12%
Always finished or no antibiotics	4,203	93%	2,646	88%	3,524	86%	2,053	90%	3,104	87%	3,361	87%
Saved antibiotics for later												
Yes, regularly	2	0.0%	7	0.2%	9	0.2%	5	0.2%	10	0.3%	6	0.2%
Yes, occasionally	37	0.8%	45	1.5%	68	1.7%	62	2.7%	60	1.7%	46	1.2%
No, never	297	7%	304	10%	492	12%	146	6%	387	11%	430	11%

Table 1. Characteristics of the HELIUS study population (N=21,617) by ethnicity *(continued)*

Variables#	Ethnicity											
	Dutch (N=4,564)		South-Asian Surinamese (N=3,043)		African Surinamese (N=4,151)		Ghanaian (N=2,339)		Turkish (N=3,614)		Moroccan (N=3,906)	
Not applicable (no antibiotics)	4,203	93%	2,646	88%	3,524	86%	2,053	91%	3,104	87%	3,361	87%
Ever asked GP for antibiotics												
Yes, regularly	38	0.8%	34	1.1%	26	0.6%	36	1.6%	67	1.9%	71	1.9%
Yes, occasionally	824	18%	491	16%	607	15%	401	18%	734	21%	634	17%
No, never	3,674	81%	2,482	83%	3,441	84%	1,835	81%	2,744	77%	3,074	81%

All variables are reported as n (%), unless otherwise indicated

† Answered "yes" to the statements below

* The Dutch General Practitioners guidelines (and those of other European countries) advise against the use of antibiotics for fever in general or sore throat, as they usually constitute viral infections, with only a few exceptions in both cases. Therefore, antibiotics are in general not appropriate for these conditions.

** Based on a summed score with cutoff determined by an Item Response Theory model (≥4 out of 5 antibiotic knowledge questions correctly answered was considered as having a higher level of knowledge)

N.A. Not applicable (categories not applicable due to Dutch ethnicity)

Missing data, *n*: marital status 128; diabetes 78; stroke 55; myocardial infarction 33; heart condition 83; malignant disorders 137; fatigue 145; high blood pressure 101; artery stenosis 140; respiratory diseases 115; bowel diseases 115; psoriasis 98; eczema 117; incontinence 126; BMI 23; smoking 107; alcohol 127; perceived health 61; AB effective for influenza 614; AB effective for pneumonia 477; AB effective for fever 685; AB effective for sore throat 625; AB effective for bronchitis 663; asked GP for AB 414; did not finish treatment 292; saved AB 325

Abbreviations: IQR – Inter Quartile Range; CVA – Cerebro Vascular Accident; MI – Myocardial infarction; N.A. – Not Applicable; GP – General Practitioner

Table 2. Variables associated with higher antibiotic knowledge in HELIUS study population (N=21,617) (logistic regression analysis)

	Univariable			Multivariable (N= 20,081*)#		
	OR	**(95% CI)**	**P-values**	**aOR**	**(95% CI)**	**P-values**
Sociodemographics						
Ethnicity			<.001			<.001
Dutch	Ref			Ref		
South-Asian Surinamese						
1st generation	0.49	0.44-0.55		0.53	0.47-0.60	
2nd generation	0.56	0.47-0.67		0.60	0.50-0.73	
African Surinamese						
1st generation	0.51	0.46-0.57		0.53	0.47-0.59	
2nd generation	0.75	0.62-0.91		0.79	0.64-0.96	
Ghanaian						
1st generation	0.31	0.27-0.34		0.31	0.27-0.35	
2nd generation	0.64	0.41-0.98		0.74	0.47-1.18	
Turkish						
1st generation	0.35	0.31-0.39		0.40	0.36-0.45	
2nd generation	0.56	0.48-0.65		0.62	0.53-0.74	
Moroccan						
1st generation	0.51	0.45-0.57		0.56	0.50-0.63	
2nd generation	0.71	0.61-0.83		0.75	0.63-0.89	
Female sex	1.18	1.11-1.25	<.001	1.32	1.23-1.40	<.001
Age			<.001			<.001
<25 years	Ref			Ref		
25-34 years	1.24	1.01-1.34		1.32	1.16-1.50	
35-44 years	0.99	0.81-1.05		1.30	1.14-1.49	
45-54 years	0.85	0.71-0.91		1.19	1.04-1.37	
55-64 years	0.95	0.78-1.02		1.26	1.08-1.45	
≥65 years	1.06	0.84-1.22		1.15	0.95-1.39	
Educational level			<.001			
Unknown	Ref					
No school/elementary school	1.10	0.79-1.55				
Lower vocational/lower secondary school	1.27	0.91-1.77				
Intermediate vocational/ intermediate secondary school	1.54	1.11-2.15				

Table 2. Variables associated with higher antibiotic knowledge in HELIUS study population (N=21,617) (logistic regression analysis) *(continued)*

	Univariable			Multivariable (N= 20,081*)#		
	OR	**(95% CI)**	**P-values**	**aOR**	**(95% CI)**	**P-values**
Higher vocational/university	2.06	1.47-2.87				
Marital status			<.001			
Married/registered partnership	Ref					
Cohabiting	1.15	1.04-1.27				
Unmarried/never married	1.08	1.01-1.16				
Divorced/separated	0.81	0.74-0.89				
Widow/widower	1.10	0.88-1.36				
Health status						
Self-reported medical conditions (previous 12 months)						
Diabetes mellitus	0.70	0.63-0.77	<.001			
CVA/one-sided loss of bodily function ≤1 day	0.85	0.75-0.97	.015			
MI incl. ≥half hour chest pain or dotter/ bypass operation	0.71	0.65-0.77	<.001	0.89	0.81-0.98	.017
Severe heart condition	0.61	0.52-0.73	<.001			
Malignant disorder	0.97	0.78-1.20	.752			
Severe or chronic fatigue	0.80	0.75-0.85	<.001	0.89	0.82-0.95	.001
High blood pressure	0.78	0.73-0.84	<.001			
Artery stenosis	0.69	0.61-0.78	<.001			
Respiratory diseases	0.68	0.62-0.75	<.001	0.80	0.72-0.88	<.001
Serious/persistent intestinal disorders	0.88	0.79-0.98	.024			
Psoriasis	0.76	0.66-0.89	.001			
(Chronic) eczema	0.93	0.84-1.02	.121			
Incontinence	0.84	0.76-0.93	.001			
Body Mass Index			<.001			.001
<18.5	Ref			Ref		
18.5-25	1.03	0.81-1.31		1.02	0.80-1.31	
25-30	0.80	0.63-1.01		0.96	0.75-1.24	
30-40	0.67	0.52-0.85		0.86	0.66-1.11	
≥40	0.58	0.43-0.78		0.78	0.57-1.08	
Smoking			<.001			
Yes	Ref					

Table 2. Variables associated with higher antibiotic knowledge in HELIUS study population (N=21,617) (logistic regression analysis) *(continued)*

	Univariable			Multivariable (N= 20,081*)#		
	OR	**(95% CI)**	**P-values**	**aOR**	**(95% CI)**	**P-values**
No, never	1.03	0.96-1.11				
No, but ever	1.19	1.09-1.30				
Alcohol usage			<.001			
Never	Ref					
Not in previous 12 months	0.89	0.80-1.00				
Monthly or less	1.10	1.01-1.20				
2-4 times per month	1.27	1.16-1.40				
2-3 times per week	1.35	1.22-1.49				
≥4 times per week	1.59	1.42-1.78				
Difficulty with Dutch language			<.001			
No	Ref					
Yes	0.65	0.61-0.69				
Not applicable	1.70	1.56-1.85				
Perceived health			<.001			
Excellent	Ref					
Very good	0.97	0.85-1.12				
Good	0.83	0.73-0.93				
Mediocre	0.63	0.56-0.72				
Bad	0.48	0.40-0.57				
Antibiotics						
Ever asked GP for antibiotics			<.001			<.001
No, never	Ref			Ref		
Yes, regularly	0.51	0.40-0.65		0.60	0.46-0.77	
Yes, occasionally	0.57	0.53-0.61		0.59	0.55-0.64	
Did not finish treatment			<.001			<.001
Always finished or no antibiotics	Ref			Ref		
Yes, regularly	0.51	0.39-0.67		0.71	0.54-0.94	
Yes, occasionally	0.73	0.66-0.80		0.80	0.73-0.88	

* Fewer observations in the multivariable model than in the total study population were due to missing observations on certain covariates

We found significant interactions between ethnicity and sex (p=0.007) and ethnicity and age (p=0.047)

Abbreviations: OR – Odds Ratio; aOR – adjusted Odds Ratio CI – Confidence Interval; CVA – Cerebro Vascular Accident; MI – Myocardial infarction; GP – General Practitioner

ing medical conditions: myocardial Infarction (MI), severe or chronic fatigue, respiratory diseases and having a BMI ≥25. Lower odds for higher level of antibiotic knowledge were also seen among individuals who regularly or occasionally requested antibiotics from their GP or who regularly or occasionally did not finish treatment.

Ethnic difference in antibiotic use

Of the 19,895 HELIUS participants consenting to link their data to other health registries, 15,461 were linked to the AHD (77.7%). Of these 15,461 participants, 15,007 (97%) were insured for ≥365 days in the year prior to their HELIUS study visit. Supplementary table 1 shows the characteristics of the study participants linked versus not linked to the AHD. Participants present in the AHD register had a lower level of education, higher prevalence of medical conditions, and less often had higher levels of antibiotic knowledge.

Table 3 describes antibiotic use according to ethnicity for participants registered in the AHD. In total, 31,530 antibiotic prescriptions were recorded over the study period. The proportion of participants receiving ≥1 antibiotic prescription in the year prior to their HELIUS study visit was highest among first-generation Turkish participants (25%) and was comparably high among second-generation Ghanaian and first-generation Moroccan participants (both 25%). The proportion of participants receiving ≥1 antibiotic prescription in the year prior to the HELIUS study visit was lowest in Dutch and second generation South-Asian Surinamese participants (both 16%).

When considering the entire period during which participants were insured at Achmea prior to the HELIUS study visit (median 6.0 years, IQR 5.0 to 6.0), the proportion of participants receiving ≥1 antibiotic prescription was highest among first generation Turkish participants (69%) and lowest in second-generation Ghanaian participants (49%). The mean number of prescriptions during the entire insured period was comparable to the mean number of prescriptions in the year prior to HELIUS study visit for all ethnic groups (table 2).

Determinants of antibiotic use and number of prescriptions

Table 4 shows the results from the analysis on the association between ethnicity and having received ≥1 antibiotic prescription in the year prior to the HELIUS study visit. Differences across ethnic groups were observed overall for any antibiotic prescription in both univariable (p<0.001) and multivariable analysis (p<0.001). In multivariable analysis, compared to Dutch individuals, first and second generation Ghanaian individuals and first-generation Moroccan individuals had significantly higher odds of receiving ≥1 antibiotic prescription. Adding variables on antibiotic-related behavior and level of antibiotic use knowledge to the multivariable model did not change these associations.

Table 3. Antibiotic use in participants linked to AHD (N=15,007) stratified by ethnicity

	Ethnicity										
	Dutch	South-Asian Surinamese		African Surinamese		Ghanaian		Turkish		Moroccan	
	(N=2,071)	1st gen (N=1,645)	2nd gen (N=452)	1st gen (N=2,334)	2nd gen (N=432)	1st gen (N=1,789)	2nd gen (N=84)	1st gen (N=2,102)	2nd gen (N=776)	1st gen (N=2,119)	2nd gen (N=857)
Duration of insurance at Achmea (in years) between 2010 and 2015, median (IQR)	6.0 (4.0-6.0)	6.0 (6.0-6.0)	6.0 (4.0-6.0)	6.0 (6.0-6.0)	6.0 (5.0-6.0)	6.0 (6.0-6.0)	6.0 (4.0-6.0)	6.0 (96.0-6.0)	6.0 (5.0-6.0)	6.0 (6.0-6.0)	6.0 (4.0-6.0)
Within year prior to HELIUS study visit											
Participants with ≥1 ABP	16%	22%	16%	17%	17%	22%	25%	25%	19%	25%	17%
Number of ABP among all participants included in the AHD											
Mean	0.26	0.39	0.26	0.28	0.28	0.33	0.55	0.40	0.34	0.41	0.28
Median (IQR)	0.00 (0.0-0.0)	0.00 (0.0-0.0)	0.00 (0.0-0.0)	0.00 (0.0-0.0)	0.00 (0.0-0.0)	0.00 (0.0-0.0)	0.00 (0.0-0.5)	0.00 (0.0-1.0)	0.00 (0.0-0.0)	0.00 (0.0-1.0)	0.00 (0.0-0.0)
Number of ABP among participants with ≥1 ABP											
Mean	1.66	1.75	1.59	1.58	1.64	1.51	2.19	1.57	1.78	1.64	1.63
Median (IQR)	1.00 (1.0-2.0)	1.00 (1.0-2.0)	1.00 (1.0-2.0)	1.00 (1.0-2.0)	1.00 (1.0-2.0)	1.00 (1.0-2.0)	2.00 (1.0-3.0)	1.00 (1.0-2.0)	1.00 (1.0-2.0)	1.00 (1.0-2.0)	1.00 (1.0-2.0)
During entire insured period											
Participants with ≥1 ABP	51%	63%	53%	56%	51%	62%	49%	69%	59%	67%	54%
Number of ABP per year among all participants included in the AHD											
Mean	0.31	0.46	0.30	0.31	0.30	0.35	0.34	0.44	0.38	0.43	0.33
Median (IQR)	0.17 (0.0-0.3)	0.17 (0. 0-0.6)	0.17 (0. 0-0.3)	0.17 (0.0-0.3)	0.17 (0.0-0.3)	0.17 (0.0-0.5)	0.00 (0.0-0.7)	0.25 (0.0-0.7)	0.17 (0.0-0.5)	0.17 (0.0-0.6)	0.17 (0.0-0.5)

Abbreviations: ABP – Antibiotic Prescription; IQR – Inter Quartile Range

Table 4. Variables associated with having received ≥1 antibiotic prescription in the year prior to HELIUS visit in participants linked to AHD (N=15,007) (logistic regression analysis)

	Univariable			Multivariable excluding variables on antibiotic-related behavior			Multivariable including variables on antibiotic-related behavior		
	OR	(95% CI)	P-values	aOR	(95% CI)	P-values	aOR	(95% CI)	P-values
Sociodemographics									
Ethnicity			<.001			<.001			.004
Dutch	Ref			Ref			Ref		
South-Asian Surinamese									
1st generation	1.57	1.33-1.85		1.05	0.86-1.27		1.04	0.85-1.26	
2nd generation	1.05	0.79-1.38		0.95	0.71-1.28		0.92	0.68-1.24	
African Surinamese									
1st generation	1.15	0.98-1.35		0.89	0.75-1.07		0.88	0.73-1.05	
2nd generation	1.14	0.87-1.50		1.02	0.76-1.36		0.96	0.71-1.29	
Ghanaian									
1st generation	1.53	1.30-1.81		1.38	1.14-1.68		1.28	1.05-1.56	
2nd generation	1.81	1.09-3.01		1.92	1.12-3.27		1.64	0.94-2.87	
Turkish									
1st generation	1.84	1.58-2.15		1.07	0.88-1.31		1.00	0.82-1.22	
2nd generation	1.27	1.02-1.57		1.02	0.80-1.30		1.01	0.79-1.29	
Moroccan									
1st generation	1.81	1.55-2.11		1.22	1.00-1.49		1.15	0.94-1.41	
2nd generation	1.14	0.92-1.41		0.93	0.73-1.19		0.89	0.69-1.14	
Female sex	1.91	1.75-2.08	<.001	1.77	1.60-1.95	<.001	1.70	1.54-1.88	<.001

Table 4. Variables associated with having received ≥1 antibiotic prescription in the year prior to HELIUS visit in participants linked to AHD (N=15,007) (logistic regression analysis) *(continued)*

	Univariable			Multivariable excluding variables on antibiotic-related behavior			Multivariable including variables on antibiotic-related behavior		
	OR	(95% CI)	P-values	aOR	(95% CI)	P-values	aOR	(95% CI)	P-values
Age			<.001						
<25 years	Ref								
25-34 years	1.04	0.87-1.24							
35-44 years	1.28	1.09-1.50							
45-54 years	1.34	1.15-1.56							
55-64 years	1.42	1.21-1.66							
≥65 years	1.59	1.29-1.96							
Educational level			<.001			.005			.001
Unknown	Ref			Ref			Ref		
No school/elementary school	1.41	0.95-2.09		1.55	0.93-2.58		1.55	0.87-2.75	
Lower vocational/lower secondary school	1.05	0.71-1.55		1.50	0.90-2.50		1.47	0.83-2.60	
Intermediate vocational/ intermediate secondary school	0.93	0.63-1.39		1.43	0.86-2.39		1.38	0.78-2.44	
Higher vocational/university	0.66	0.44-0.99		1.18	0.71-1.99		1.12	0.63-2..00	
Marital status			<.001						
Married/registered partnership	Ref								
Cohabiting	0.66	0.56-0.77							
Unmarried/never married	0.78	0.71-0.86							
Divorced/separated	1.14	1.02-1.27							
Widow/widower	1.26	0.98-1.64							

Table 4. Variables associated with having received ≥1 antibiotic prescription in the year prior to HELIUS visit in participants linked to AHD (N=15,007) (logistic regression analysis) *(continued)*

	Univariable			Multivariable excluding variables on antibiotic-related behavior			Multivariable including variables on antibiotic-related behavior		
	OR	(95% CI)	P-values	aOR	(95% CI)	P-values	aOR	(95% CI)	P-values
Health status									
Self-reported medical conditions (previous 12 months)									
Diabetes mellitus	1.75	1.56-1.96	<.001	1.29	1.13-1.47	<.001	1.30	1.13-1.48	<.001
CVA/one-sided loss of bodily function ≤1 day	1.38	1.18-1.62	<.001						
MI incl. ≥half hour chest pain or dotter/bypass operation	1.78	1.60-1.98	<.001	1.28	1.13-1.45	<.001	1.24	1.09-1.40	.001
Severe heart condition	1.45	1.18-1.79	.001						
Malignant disorder	1.93	1.52-2.47	<.001	1.33	1.02-1.74	.037			
Severe or chronic fatigue	1.86	1.71-2.02	<.001	1.20	1.08-1.33	.001	1.16	1.04-1.29	.008
High blood pressure	1.36	1.25-1.49	<.001						
Artery stenosis	1.46	1.25-1.70	<.001	0.80	0.67-0.96	.014	0.77	0.64-0.92	.004
Respiratory diseases	2.19	1.96-2.44	<.001	1.66	1.47-1.87	<.001	1.59	1.41-1.81	<.001
Serious/persistent intestinal disorders	1.87	1.64-2.12	<.001	1.24	1.07-1.43	.004	1.22	1.05-1.41	.009
Psoriasis	1.28	1.05-1.56	.015						
(Chronic) eczema	1.30	1.15-1.47	<.001						
Incontinence	2.08	1.85-2.35	<.001	1.32	1.15-1.52	<.001	1.32	1.15-1.52	<.001
Body Mass Index			<.001						
<18.5	Ref								
18.5-25	1.03	0.72-1.46							
25-30	1.24	0.87-1.76							

Table 4. Variables associated with having received ≥1 antibiotic prescription in the year prior to HELIUS visit in participants linked to AHD (N=15,007) (logistic regression analysis) *(continued)*

	Univariable			Multivariable excluding variables on antibiotic-related behavior			Multivariable including variables on antibiotic-related behavior		
	OR	(95% CI)	P-values	aOR	(95% CI)	P-values	aOR	(95% CI)	P-values
30-40	1.64	1.15 -2.33							
≥40	1.97	1.31-2.97							
Smoking			.184			<.001			.003
Yes	Ref			Ref			Ref		
No, never	0.99	0.90-1.09		0.78	0.69-0.87		0.82	0.72-0.92	
No, but ever	0.90	0.80-1.02		0.91	0.79-1.04		0.93	0.81-1.07	
Alcohol usage			<.001			.017			.012
Never	Ref			Ref			Ref		
Not in previous 12 months	0.88	0.77-1.02		0.96	0.82-1.12		0.95	0.81-1.12	
Monthly or less	0.68	0.61-0.77		0.83	0.72-0.95		0.82	0.71-0.94	
2-4 times per month	0.73	0.64-0.83		0.93	0.79-1.09		0.92	0.78-1.08	
2-3 times per week	0.64	0.55-0.75		0.92	0.76-1.10		0.89	0.74-1.08	
≥4 times per week	0.48	0.39-0.58		0.70	0.55-0.88		0.68	0.54-0.86	
Difficulty with Dutch language			<.001						
No	Ref								
Yes	1.32	1.21-1.43							
Not applicable	0.80	0.71-0.91							
Perceived health			<.001			<.001			.002
Excellent	Ref			Ref			Ref		

Table 4. Variables associated with having received ≥1 antibiotic prescription in the year prior to HELIUS visit in participants linked to AHD (N=15,007) (logistic regression analysis) *(continued)*

	Univariable			Multivariable excluding variables on antibiotic-related behavior			Multivariable including variables on antibiotic-related behavior		
	OR	**(95% CI)**	**P-values**	**aOR**	**(95% CI)**	**P-values**	**aOR**	**(95% CI)**	**P-values**
Very good	1.09	0.86-1.37		1.05	0.83-1.34		1.03	0.80-1.31	
Good	1.55	1.27-1.90		1.22	0.99-1.51		1.20	0.97-1.49	
Mediocre	2.47	2.01-3.04		1.37	1.09-1.72		1.32	1.05-1.67	
Bad	3.85	3.02-4.91		1.69	1.28-2.23		1.59	1.20-2.11	
Antibiotic-related behavior									
Higher antibiotic knowledge			<.001						
No	Ref								
Yes	0.77	0.71-0.84							
Ever asked GP for antibiotics			<.001						<.001
No, never	Ref						Ref		
Yes, regularly	4.72	3.59-6.21					3.07	2.28-4.14	
Yes, occasionally	2.42	2.20-2.66					2.11	1.91-2.34	
Did not finish treatment			<.001						<.001
Always finished or no antibiotics	Ref						Ref		
Yes, regularly	2.70	2.02-3.62					1.80	1.29-2.50	
Yes, occasionally	1.62	1.45-1.83					1.32	1.16-1.50	

Abbreviations: OR – Odds Ratio; aOR – adjusted Odds Ratio; CI – Confidence Interval; CVA – Cerebro Vascular Accident; MI – Myocardial infarction; GP – General Practitioner

Table 5 shows the results from the analysis on the association between ethnicity and total number of antibiotic prescriptions received in the year prior to the HELIUS study visit. Differences across ethnic groups were observed overall for the number of antibiotic prescriptions in both univariable and multivariable analysis (both p=0.004). First-generation African Surinamese and Turkish migrants had a significantly lower number of antibiotic prescriptions compared to individuals of Dutch origin. Only second-generation Ghanaian participants has more prescriptions compared to Dutch participants. Furthermore, female sex, diabetes mellitus, MI, malignant disorder, respiratory disease, eczema and worse perceived health were significantly associated with a higher number of antibiotic prescriptions.

Having a higher level of antibiotic knowledge was not significantly associated with the number of prescriptions when included in multivariable analysis (p=0.446). No significant interactions between ethnicity and sex or education were observed. Finally, adjusting the association between ethnicity and antibiotic use for antibiotic-related behaviors did not change these associations.

DISCUSSION

Our study shows that knowledge on the need to use antibiotics for treatment is lower among all ethnic minority groups compared to Dutch, with second generation ethnic minorities showing higher levels of knowledge compared to first generation migrants. We also observed ethnic differences in the use of antibiotics, with a higher proportion having received at least one prescription, but a lower mean number of antibiotic prescriptions among some ethnic minority groups compared to Dutch. The only ethnic group with a significantly higher number of antibiotic prescriptions was second generation Ghanaian participants. Furthermore, we showed that a lower level of antibiotic knowledge was not associated with receiving antibiotics or average number of antibiotic prescriptions, and that ethnic differences in antibiotic use therefore cannot be explained by level of knowledge on antibiotics.

A previous study in Dutch primary care centers demonstrated higher use of antibiotics among first-generation migrants from Turkey, Morocco, Surinam or the Antilles compared to Dutch, after adjustment for age, sex, education, presence of chronic diseases, and smoking.(3) We found that the odds of having ≥1 antibiotic prescription was higher in some ethnic groups in unadjusted analysis, but after adjusting for several variables including medical conditions, the odds were significantly higher among Ghanaian and first-generation Moroccan participants only. In contrast, in our analyses on the number

Table 5. Variables associated with number of antibiotic prescriptions in participants linked to ADH (N=15,007) (zero-inflated negative binomial regression analysis)

	Univariable*			Multivariable excluding variables on antibiotic-related behavior			Multivariable including variables on antibiotic-related behavior		
	IRR	**(95% CI)**	**P-values**	**IRR**	**(95% CI)**	**P-values**	**IRR**	**(95% CI)**	**P-values**
Sociodemographics									
Ethnicity			.004			.004			.001
Dutch	Ref			Ref			Ref		
South-Asian Surinamese									
1st generation	1.06	0.85-1.31		0.86	0.68-1.10		1.02	0.82-1.28	
2nd generation	0.82	0.55-1.21		0.94	0.61-1.44		0.94	0.64-1.36	
African Surinamese									
1st generation	0.79	0.64-0.99		0.73	0.58-0.93		0.81	0.65-1.00	
2nd generation	0.83	0.57-1.22		0.90	0.59-1.36		0.93	0.65-1.33	
Ghanaian									
1st generation	0.75	0.60-0.94		0.77	0.60-1.00		0.81	0.65-1.02	
2nd generation	1.66	0.89-3.11		2.09	1.06-4.12		2.70	1.47-4.94	
Turkish									
1st generation	0.85	0.69-1.04		0.74	0.59-0.92		0.79	0.64-0.97	
2nd generation	1.14	0.87-1.51		1.14	0.84-1.53		1.10	0.84-1.44	
Moroccan									
1st generation	0.99	0.81-1.21		0.89	0.70-1.11		0.89	0.72-1.10	
2nd generation	0.84	0.63-1.13		0.92	0.67-1.27		1.02	0.76-1.37	
Female sex	1.36	1.20-1.54	<.001	1.35	1.18-1.54	<.001	1.29	1.15-1.46	<.001

Table 5. Variables associated with number of antibiotic prescriptions in participants linked to ADH (N=15,007) (zero-inflated negative binomial regression analysis) *(continued)*

	Univariable*			Multivariable excluding variables on antibiotic-related behavior			Multivariable including variables on antibiotic-related behavior		
	IRR	(95% CI)	P-values	IRR	(95% CI)	P-values	IRR	(95% CI)	P-values
Age			.023						
<25 years	Ref								
25-34 years	1.09	0.91-1.30							
35-44 years	1.16	0.98-1.38							
45-54 years	1.09	0.92-1.29							
55-64 years	1.14	0.95-1.36							
≥65 years	1.44	1.16-1.80							
Educational level			.096						
Unknown	Ref								
No school/elementary school	1.74	0.86-3.52							
Lower vocational/lower secondary school	1.74	0.86-3.52							
Intermediate vocational/ intermediate secondary school	1.57	0.77-3.17							
Higher vocational/university	1.45	0.71-2.96							
Marital status			.212						
Married/registered partnership	Ref								
Cohabiting	0.85	0.72-1.00							
Unmarried/never married	0.99	0.89-1.10							
Divorced/separated	1.01	0.90-1.13							
Widow/widower	1.14	0.90-1.45							

Table 5. Variables associated with number of antibiotic prescriptions in participants linked to ADH (N=15,007) (zero-inflated negative binomial regression analysis) *(continued)*

	Univariable*			Multivariable excluding variables on antibiotic-related behavior			Multivariable including variables on antibiotic-related behavior		
	IRR	(95% CI)	P-values	IRR	(95% CI)	P-values	IRR	(95% CI)	P-values
Health status									
Self-reported medical conditions (previous 12 months)									
Diabetes mellitus	1.34	1.17-1.54	<.001	1.21	1.04-1.41	.015	1.22	1.06-1.41	.005
CVA/one-sided loss of bodily function ≤1 day	1.10	0.95-1.29	.208						
MI incl. ≥half hour chest pain or dotter/bypass operation	1.35	1.19-1.54	<.001	1.22	1.06-1.41	.005			
Severe heart condition	1.26	1.04-1.52	.019						
Malignant disorder	1.91	1.47-2.48	<.001	1.60	1.21-2.12	.001	1.60	1.28-2.00	<.001
Severe or chronic fatigue	1.29	1.15-1.44	<.001						
High blood pressure	1.14	1.04-1.25	.006						
Artery stenosis	1.30	1.08-1.57	.005						
Respiratory diseases	1.50	1.32-1.71	<.001	1.34	1.16-1.54	<.001	1.29	1.13-1.47	<.001
Serious/persistent intestinal disorders	1.24	1.07-1.44	.004						
Psoriasis	1.08	0.90-1.31	.406						
(Chronic) eczema	1.21	1.07-1.37	.002	1.14	1.00-1.29	.042			
Incontinence	1.31	1.13-1.50	<.001						
Body Mass Index			.736						
<18.5	Ref								
18.5-25	0.98	0.70-1.37							
25-30	0.98	0.70-1.37							

Table 5. Variables associated with number of antibiotic prescriptions in participants linked to ADH (N=15,007) (zero-inflated negative binomial regression analysis) *(continued)*

	Univariable*			Multivariable excluding variables on antibiotic-related behavior			Multivariable including variables on antibiotic-related behavior		
	IRR	(95% CI)	P-values	IRR	(95% CI)	P-values	IRR	(95% CI)	P-values
30-40	1.04	0.74-1.46							
≥40	1.06	0.71-1.57							
Smoking			.099						
Yes	Ref								
No, never	1.13	1.00-1.29							
No, but ever	1.02	0.87-1.21							
Alcohol usage			.075						
Never	Ref								
Not in previous 12 months	0.93	0.77-1.13							
Monthly or less	0.94	0.80-1.11							
2-4 times per month	1.01	0.85-1.20							
2-3 times per week	0.73	0.58-0.91							
≥4 times per week	0.81	0.61-1.08							
Difficulty with Dutch language			.320						
No	Ref								
Yes	0.94	0.86-1.04							
Not applicable	1.05	0.88-1.25							
Perceived health			<.001			.001			<.001
Excellent	Ref			Ref			Ref		

Table 5. Variables associated with number of antibiotic prescriptions in participants linked to ADH (N=15,007) (zero-inflated negative binomial regression analysis) *(continued)*

	Univariable*			Multivariable excluding variables on antibiotic-related behavior			Multivariable including variables on antibiotic-related behavior		
	IRR	(95% CI)	P-values	IRR	(95% CI)	P-values	IRR	(95% CI)	P-values
Very good	0.98	0.68-1.42		1.03	0.71-1.49		0.99	0.70-1.39	
Good	1.29	0.94-1.77		1.19	0.85-1.65		1.17	0.86-1.59	
Mediocre	1.73	1.25-2.39		1.40	1.00-1.97		1.44	1.05-1.97	
Bad	2.30	1.62-3.26		1.71	1.17-2.49		1.72	1.22-2.43	
Antibiotic-related behavior									
Higher antibiotic knowledge			.054						
No	Ref								
Yes	0.92	0.85-1.00							
Ever asked GP for antibiotics			<.001						<.001
No, never	Ref						Ref		
Yes, regularly	2.96	2.34-3.73					1.87	1.45-2.41	
Yes, occasionally	1.75	1.59-1.92					1.15	1.06-1.30	
Did not finish treatment			<.001						
Always finished or no antibiotics	Ref								
Yes, regularly	1.50	1.12-2.00							
Yes, occasionally	1.32	1.18-1.48							

* Accounts for zero-inflated distribution

Abbreviations: IRR – Incidence Risk Ratio; CI – Confidence Interval; CVA – Cerebro Vascular Accident; MI – Myocardial infarction; GP – General Practitioner

of antibiotic prescriptions as an outcome, only second-generation Ghanaian migrants were at higher risk of receiving a higher number of prescriptions compared to Dutch participants. For all other ethnic groups, no evidence of a higher risk for more frequent prescriptions was found, while even a lower number was present for first-generation African Suriname and Turkish participants. To the best of our knowledge, no other studies have evaluated the variation in level of antibiotic knowledge and antibiotic use between ethnic groups and thus our findings need to be confirmed. Notably, our findings on antibiotic prescriptions and ethnicity are in line with a large retrospective cohort study performed in pediatric emergency departments in the United States.(15) This study also looked at the association between ethnicity and antibiotic prescribing, showing that other ethnic groups received less antibiotics for viral infections than non-Hispanic white children

Lower odds for higher level of antibiotic use knowledge were also seen among individuals who regularly or occasionally requested antibiotics from their GP or who regularly or occasionally did not finish treatment. These findings suggest that improving antibiotic knowledge might decrease the number of requests for antibiotics in primary care and improve appropriate use.

Our study has several strengths. First, the HELIUS study consists of a large number of participants from major ethnic groups living in the same city, with representation from all socioeconomic levels. Second, all outcomes and determinants were measured using the same methodology across all ethnic groups and HELIUS used translated questionnaires and had ethnically-matched interviewers and research assistants to provide assistance during data collection. These procedures enhance the comparability between ethnic groups. Another major strength of the current study is that HELIUS data could be linked to data from a health insurance register covering the majority (77.7%) of the study population.

Our study has also limitations. First, although HELIUS participants were recruited via an ethnicity-stratified random selection of the municipal registry of Amsterdam, the response rate for HELIUS study was 28% and there may be selection bias.(10) However, analysis from a previous HELIUS study have shown that participants are not exceedingly different from non-respondents regarding sociodemographic variables.(10) Second, we did not take into account the use of antibiotics purchased over the counter in the home country of participants (6, 16-18), and we might therefore have underestimated antibiotic use in non-Dutch ethnic groups. As a recent HELIUS study found that Dutch people of Turkish or Moroccan origin were more likely to use healthcare in the Netherlands as well as their country of origin,(19) underestimation of antibiotic use in non-Dutch ethnic

groups seems unlikely. Third, since several characteristics, such as education level and medical conditions, of HELIUS participants insured at Achmea differed from those insured elsewhere, selection bias could have been introduced in analysis on antibiotic use. This difference could be due to the fact that the City of Amsterdam provided health insurance discounts with Achmea for low-income individuals. These differences were corrected for during multivariable analyses to the most possible extent. Fourth, the variable 'ever asked GP for antibiotics' does not discriminate between appropriate or inappropriate requests for antibiotics and misclassification might have occurred. However, this variable gives some information on participants' attitudes towards antibiotic use. Furthermore, due to privacy restrictions, we were unable to include indication for antibiotic therapy and duration of antibiotic use as additional indices for antibiotic use (apart from the number of antibiotics prescribed). Moreover, since this was a cross-sectional study, we were unable to model antibiotic knowledge with future antibiotic prescriptions. Further research should examine the association of antibiotic knowledge with future antibiotic prescriptions. Finally, we are unable to determine if individuals were more demanding towards their GP or if their GPs were more lenient in prescribing antibiotics during illness.(4, 5) Neither completing antibiotic therapy, assessed by pill count, nor duration of antibiotic use could be taken into account as these data were not available.

Conclusions

To our knowledge, this study is the first to examine ethnic disparities in level of antibiotic knowledge and use in a large population-based sample among adults with different ethnic backgrounds. Health policy makers and healthcare professionals are increasingly developing interventions to improve the quality of antibiotic use, which is needed to help contain antimicrobial resistance. Targeted campaigns can be considered, for instance, during the annual European Antibiotic Awareness Day, since this event addresses improvement in the quality of antibiotic use to the general public.(20) Still, this study shows that a lower level of antibiotic knowledge is not necessarily linked to higher antibiotic usage, indicating that interventions aimed at improving knowledge alone might be insufficient to reduce antibiotic use. Nevertheless, the underlying reasons for these findings need further evaluation.

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Conflicts of interest

The authors declare that they have no competing interests.

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Supplementary table 1. Characteristics of participants not linked versus linked to the Achmea Health Database

	Non-linked participants (N=6,956)		Linked participants (N=15,007)		P-value
	n	%	n	%	
Sociodemographics					
Ethnicity					<.001
Dutch	2,493	35%	2,071	14%	
South-Asian Surinamese	946	13%	2,097	14%	
African Surinamese	1,385	19%	2,766	18%	
Ghanaian	466	7%	1,873	12%	
Turkish	736	10%	2,878	19%	
Moroccan	930	13%	2,976	20%	
Other/unknown	202	2.8%	346	2.3%	
Female sex	4,041	56%	8,769	58%	.005
Median age in years (IQR)	44	(32-54)	46	(35-55)	<.001
Migration generation					<.001
1st generation	3,436	48%	10,283	69%	
2nd generation	1,229	17%	2,653	18%	
Not applicable	2,493	35%	2,071	14%	
Educational level					<.001
Unknown	50	0.7%	157	1.1%	
No school/elementary school	621	9%	3,255	22%	
Lower vocational/lower secondary school	1,370	19%	4,433	30%	
Intermediate vocational/ intermediate secondary school	1,998	28%	4,423	29%	
Higher vocational/university	3,119	44%	2,739	18%	
Marital status					<.001
Married/registered partnership	2,689	38%	5,910	40%	
Cohabiting	1,031	14%	1,377	9%	
Unmarried/never married	2,559	36%	4,929	33%	
Divorced/ separated	751	11%	2,357	16%	
Widow/widower	106	1.5%	328	2.2%	
Health status					
Self-reported medical conditions (previous 12 months)					
Diabetes mellitus	396	6%	1,606	11%	<.001
CVA/one-sided loss of bodily function ≤1 day	304	4.3%	837	6%	<.001

Supplementary table 1. Characteristics of participants not linked versus linked to the Achmea Health Database *(continued)*

	Non-linked participants (N=6,956)		Linked participants (N=15,007)		P-value
	n	%	n	%	
MI incl. ≥half hour chest pain or dotter/bypass operation	610	9%	1,902	13%	<.001
Severe heart condition	126	1.8%	467	3.1%	<.001
Malignant disorder	122	1.7%	301	2.0%	.120
Severe or chronic fatigue	1,596	22%	4,395	30%	<.001
High blood pressure	1,147	16%	3,378	23%	<.001
Artery stenosis	234	3.3%	907	6%	<.001
Respiratory diseases	602	8%	1,708	11%	<.001
Serious/persistent intestinal disorders	495	7%	1,231	8%	.001
Psoriasis	238	3.3%	560	3.8%	.124
(Chronic) eczema	698	10%	1,532	10%	.265
Incontinence	523	7%	1,374	9%	<.001
Median Body Mass Index (kg/m2) (IQR)	25.2	(22.6-28.5)	26.9	(23.9-30.6)	<.001
Smoking					<.001
Yes	1,734	24%	3,568	24%	
No, never	3,735	52%	8,600	58%	
No, but ever	1,664	23%	2,757	18%	
Alcohol usage					<.001
Never	2,090	29%	6,927	46%	
Not in previous 12 months	439	6%	1,361	9%	
Monthly or less	1,123	16%	2,457	16%	
2-4 times per month	1,238	17%	1,817	12%	
2-3 times per week	1,221	17%	1,322	9%	
≥4 times per week	1,024	14%	1,019	7%	
Difficulty with Dutch language	1,314	18%	5,857	39%	<.001
Perceived health					<.001
Excellent	629	9%	922	6%	
Very good	1,539	22%	2,020	14%	
Good	3,755	53%	7,662	51%	
Fair	1,064	15%	3,605	24%	
Poor	154	2.2%	754	5%	

Supplementary table 1. Characteristics of participants not linked versus linked to the Achmea Health Database *(continued)*

	Non-linked participants (N=6,956)		Linked participants (N=15,007)		P-value
	n	%	n	%	
Antibiotics					
Knowledge concerning antibiotics					
Antibiotics effective for influenza	909	13%	2,690	19%	<.001
Antibiotics effective for pneumonia	5,810	82%	10,816	74%	<.001
Antibiotics effective for fever	1,224	18%	2,879	20%	<.001
Antibiotics effective for sore throat	1,574	22%	3,993	27%	<.001
Antibiotics effective for bronchitis	3,263	47%	6,123	42%	<.001
Higher antibiotic knowledge*	5,050	73%	9,593	67%	<.001
Did not finish treatment					<.001
Yes, regularly	48	0.7%	193	1.3%	
Yes, occasionally	656	9%	1,618	11%	
Saved antibiotics for later					<.001
Yes, regularly	6	0.1%	33	0.2%	
Yes, occasionally	97	1.4%	224	1.5%	
Not applicable	6,396	90%	12,962	88%	
Ever asked GP for antibiotics					.001
Yes, regularly	62	0.9%	212	1.4%	
Yes, occasionally	1,198	17%	2,568	17%	

* Based on a summed score with cutoff determined by an Item Response Theory model (≥4 out of 5 antibiotic knowledge questions correctly answered was considered higher level of knowledge)

Missing observations on the following variables: marital status 128; diabetes 78; stroke 97; myocardial infarction 193; heart condition 83; malignant disorders 137; migraine 113; fatigue 145; high blood pressure 101; artery stenosis 140; respiratory diseases 115; bowel diseases 115; psoriasis 98; eczema 117; incontinence 126; BMI 23; smoking 107; alcohol 127; perceived health 61; AB effective for influenza 614; AB effective for pneumonia 477; AB effective for fever 685; AB effective for sore throat 625; AB effective for bronchitis 663; higher antibiotic knowledge 958; asked GP for AB 414; did not finish treatment 292; saved AB 325

Abbreviations: IQR – Inter Quartile Range; CVA – Cerebro Vascular Accident; MI – Myocardial infarction; GP – General Practitioner

SUPPLEMENTARY METHODS

Determining threshold to define knowledge level of antibiotic use

In order to define antibiotic knowledge, we used an approach based on item response theory (IRT) (1). In brief, IRT allows a more precise definition of the relationship between a given measurement process (defined by questions related to correct antibiotic use for specific illnesses) and a latent trait (defined as 'higher' or 'lower' antibiotic knowledge). The latent space is assumed to be unidimensional (i.e. only one latent trait is being measured) and conditional independence is assumed between item responses.

The probability of an individual to correctly answer a question on antibiotic use is in function of the latent trait, ϑ, and the item parameters. Each item has two parameters: discrimination (correlation between correct item response and ϑ, with larger values representing capacity to differentiate between low and high levels of the latent trait) and difficulty (describing the location of an item with respect ϑ, with larger values indicating that the expected probability of a correct answer corresponds to higher levels of the latent trait).

We first modeled discrimination and difficulty parameters (defined as a and b, respectively) for each binary item using a one-parameter logistic model, whereby a is shared across all items and b is allowed to vary between items. This model was compared to a two-parameter logistic model, whereby both a and b are allowed to vary between items, using a likelihood-ratio test. After selecting the appropriate IRT logistic model, the probability of correctly answering each individual item was plotted in function of ϑ [defined as an item characteristic curve (ICC)].

We created an overall knowledge score on antibiotic use by summing the total number of correct responses, resulting in a score ranging from 0-5. In order to plot the expected score across levels of ϑ, a total characteristic curve (TCC) was constructed from summing the ICCs across all items. We defined higher" and "lower" antibiotic knowledge as a score corresponding to a $\vartheta = 0$. The analysis was carried out using the "irt" commands in Stata (v15.0, College Station, TX).

Reference:

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SUPPLEMENTARY RESULTS

Threshold for higher knowledge of antibiotic use

The one-parameter IRT logistic model resulted in discrimination parameter a = 0.54 (95%CI= 0.51, 0.57) and difficulty parameter b = -3.16 (95%CI=-3.32, -2.99) for the item on influenza, -2.84 (95%CI=-2.99, -2.69) for fever, -2.34 (95%CI=-2.47, -2.21) for pneumonia, -2.08 (95%CI=-2.20, -1.96) for sore throat, and -0.50 (95%CI=-0.56, -0.44) for bronchitis.

The two-parameter IRT logistic model resulted in the following discrimination and difficulty parameters, respectively: a = -0.75 (95%CI=-0.81, -0.69) and b = 1.77 (95%CI=1.64, 1.91) for the item on pneumonia, a = 0.62 (95%CI=0.57, 0.67) and b = -0.44 (95%CI=-0.49, -0.38) for bronchitis, a = 1.53 (95%CI=1.44, 1.62) and b = -0.96 (95%CI=-1.00, -0.92) for sore throat a = 2.05 (95%CI=1.91, 2.18) and b = -1.25 (95%CI=-1.30, -1.21) for influenza, a = 2.25 (95%CI=2.09, 2.41) and b = -1.09 (95%CI=-1.13, -1.05) for fever.

The two-parameter IRT logistic model was tested against the one-parameter model, with the former demonstrating better fit according to the likelihood ratio test (p<0.001). The resulting ICC is shown in Figure S1A. Given the ICC, the item on antibiotic use during pneumonia would not be regarded as useful in determining the latent trait of this study population. Nevertheless, this question remained in the composite score in order to be consistent with previous studies. The resulting TCC is shown in Figure S1B, a score of 4.02 (rounded to 4) or higher corresponds to a $\vartheta \geq 0$ and hence was determined as the threshold in defining “higher” knowledge on antibiotic use.

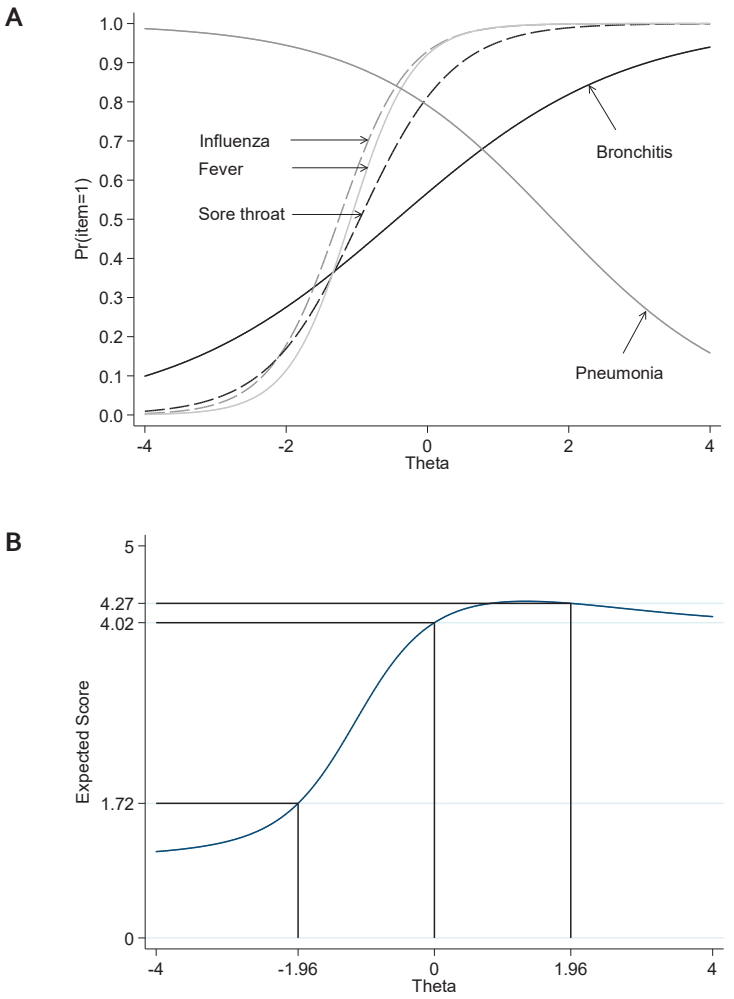

Figure S1. Item characteristic curve (A) and total characteristic curve (B) on knowledge of antibiotic use
In the item characteristic curve (**A**), the probability of answering an item correctly, Pr(item=1), is plotted against levels of the latent trait, theta. Item parameters can be obtained from this graph, with discrimination being the instantaneous slope and difficulty the corresponding theta when Pr(item=1) is 0.5. In the total characteristic curve (**B**), the expected score is plotted against levels of the latent trait, theta.

3

Measuring antibiotic knowledge using a three-item questionnaire in different ethnic groups of the general population: a psychometric analysis in the HELIUS study

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Submitted for publication

ABSTRACT

Background

We evaluated the psychometric properties of a questionnaire assessing antibiotic knowledge across ethnic groups in Amsterdam, the Netherlands.

Methods

Participants from the HELIUS study were asked five questions about appropriate use of antibiotics for certain diseases (i.e. bronchitis, influenza, sore throat, fever, and pneumonia). We studied parameters of a unidimensional latent variable model using item response theory (IRT) to describe the answers given by participants. We assessed differential item functioning (DIF) between ethnicity and criterion validity of the weighted-sumscore with antibiotic use behaviors.

Results

We included the following ethnic groups: Dutch (n=4,641), South-Asian Surinamese (n=3,369), African Surinamese (n=4,458), Ghanaian (n=2,484), Turkish (n=4,067), and Moroccan (n=4,337). Two items (bronchitis and pneumonia) were removed because of poor fit. Using the remaining three items, the IRT model demonstrated adequate fit, suggesting that antibiotic knowledge can be expressed as a unidimensional latent trait. DIF was observed for all three items. Antibiotic knowledge was associated with other behaviors of antibiotic use, both overall and across ethnic groups.

Conclusions

A 3-item questionnaire on antibiotic use for influenza, sore throat, fever is valid to measure antibiotic knowledge in large-scale studies. Item function is different between ethnicities, whose effect on content validity is minimal.

BACKGROUND

Antibiotic resistance (ABR) is a growing worldwide problem and has been stressed as a major health threat for the coming decade by the World Health Organization (WHO) (1, 2). ABR is mainly driven by an increase in antibiotic use (1). Antibiotic overuse (e.g. due to overprescribing or antibiotic prophylaxis), inappropriate prescribing, antibiotic misuse (e.g. when patients self-medicate with leftover antibiotics from previous prescriptions) and extensive agricultural use are associated with ABR emergence (3-7).

Individuals who lack knowledge on when to appropriately use antibiotics have been shown to more frequently receive antibiotic prescriptions from a physician (8-10). Insufficient knowledge about antibiotics and ABR among physicians, fear of complications and the tendency to be compliant with patient wishes can also lead to inappropriate prescribing of antibiotics (11). Considering the role of inappropriate prescribing practices on ABR, it is unsurprising that the WHO has listed improving awareness and understanding of antimicrobial resistance as one of the pillars for their Global Action Plan on antimicrobial resistance (12).

Currently, there are no validated measures of antibiotic knowledge. Such instruments could be helpful to gauge whether certain groups do not know or understand appropriate use of antibiotics and could benefit from public health campaigns to improve knowledge and understanding of antibiotic use, eventually reducing inappropriate use. We initially used a 5-item questionnaire on the perceived necessity for antibiotic treatment for several diseases to determine antibiotic knowledge in six ethnic groups in Amsterdam, the Netherlands using item response theory (IRT) (13). This study showed that antibiotic knowledge was lower among all ethnic minority groups compared to those of Dutch origin, with second-generation ethnic minorities showing higher levels of knowledge compared to first-generation migrants. The aim of the current study was to determine the difficulty and discrimination of these questions as a means to evaluate antibiotic knowledge in a large population-based cohort study conducted in Amsterdam, the Netherlands. Additionally, we evaluated differential item functioning (DIF) between ethnic groups (i.e. Dutch, South-Asian Surinamese, African Surinamese, Ghanaian, Turkish and Moroccan) and assessed criterion-validity of this questionnaire using responses to questions on antibiotic behaviors.

METHODS

Participants and design

We included participants from the HELIUS study, which is a multi-ethnic cohort study conducted in Amsterdam, the Netherlands, focusing on cardiovascular disease, mental health, and infectious diseases (14, 15). Briefly, a random sample of individuals of Dutch, Surinamese, Ghanaian, Moroccan and Turkish origin aged 18 to 70 years was obtained from the municipality register of Amsterdam and invited to participate. Participants filled in an extensive questionnaire and underwent physical examination during which biological samples were obtained. Participants who were unable to complete the questionnaire themselves were offered assistance from a trained ethnically matched interviewer. In total, 24,789 individuals were included between 2011 and 2015, of which 23,942 filled in the questionnaire. The HELIUS study was approved by the AMC Ethical Review Board. All participants provided written informed consent.

Variables measured

Ethnicity

Participants were considered to be of non-Dutch ethnic origin if: (1) they were born abroad and at least one parent was born abroad (first-generation) or (2) they were born in the Netherlands but both their parents were born abroad (second-generation). Participants of Dutch origin were born in the Netherlands themselves and both parents were born in the Netherlands. After administration of the questionnaire, the Surinamese group was further classified according to self-reported ethnic origin into “South-Asian Surinamese”, “African Surinamese”, “Javanese Surinamese” and “other/unknown Surinamese”.

Antibiotic knowledge

Participants responded to questions about appropriate use of antibiotics with respect to certain diseases, as determined from the literature and expert opinion (8, 10, 15). Individuals were asked whether they thought physicians would find it necessary to prescribe antibiotics for the following symptoms: bronchitis (yes/no), influenza-like illness (yes/no), sore throat (yes/no), fever (yes/no) and pneumonia (yes/no). Correct responses to these questions are given in Supplementary Table 1. To avoid influencing knowledge as an outcome, no information was provided by the research team beforehand regarding appropriate antibiotic use.

Antibiotic use behaviors

We asked participants on the frequency of requesting antibiotics from a general practitioner (GP) (never, regularly, occasionally, or missing). We also asked the frequency of not finishing their antibiotic treatment (never, regularly, occasionally, or missing). For individuals who responded "regularly" or "occasionally" not finishing antibiotic treatment, an additional question was asked on saving antibiotic treatment for later use (never, regularly, occasionally, missing). In analysis, individuals who reported "never" to the question of not finishing antibiotic treatment were considered as "never" saving antibiotic treatment. The full questions are provided in Supplementary Table 2.

Statistical analysis

Statistical analysis was carried out using R (v3.6.3, Vienna, Austria).

Item response model

Response y to item i from individual j is either correct ($y_{ij} = 1$)or incorrect ($y_{ij} = 0$. Items with missing values were assumed to be incorrect. To understand how responses from the items on correct antibiotic use relate to antibiotic knowledge as an underlying latent trait, we constructed a two-parameter model based on IRT (16). We estimated the conditional probability of observed y_{ij} given the latent trait η_j as:

$$\text{logit}\{\Pr(y_{ij} = 1|\eta_j)] = a_i(\eta_j + b_{ij}$$

where b_i is the item difficulty parameter and a_i is the item discrimination parameter. We fit this model via maximum likelihood estimation using an expectation-maximization approach with the "mirt" package (17). Parameter estimates were obtained using the mirt() function.

Model construction

To determine which items were to be placed in the IRT model, we constructed an initial IRT model with all 5 items (Supplementary Table 3). We removed the item with the lowest discrimination (i.e. on pneumonia) and reran the IRT model. The model with 4-items had a significantly better fit based on the log-likelihood ratio test (χ^2=26,257.13, d.f.=14, p<0.0001). We again removed the item with the lowest discrimination (i.e. on bronchitis) and obtained a 3-item IRT model demonstrating a better fit (log-likelihood ratio test compared to the 4-item model: χ^2=31,336.00, d.f.=6, p<0.0001). The 3-item IRT model containing questions on influenza-like illness, sore throat, and fever was considered the final IRT model in analysis.

IRT models assume that the joint probability of correct responses to an item pair is the product of the probabilities of correct responses to the two items, given the value of the latent variable (i.e. local independence). We tested for violations of local independence between item pairs of the final IRT model using the signed χ^2 and Cramer's V statistics (18), as calculated by the residuals() function of the "mirt" package.

Furthermore, we used the likelihood-based G2 statistic (19) to assess goodness-of-fit of the final IRT model. We used the root-square mean of error approximation ($RMSEA_2$) to assess for goodness of approximation (20). Model fit was determined as adequate if $0.05 \leq RMSEA_2 < 0.089$ and close if $RMSEA_2 < 0.05$.

Differential item functioning

DIF is present if individuals from different ethnic groups have different probabilities of responding correctly to an item despite having the same latent trait. We compared an IRT model with interaction between item parameters and ethnicity to a model without interaction using log-likelihood ratio tests. We used an auxiliary DIF test approach (21), whereby one test statistic is observed for a single studied item and all other items are anchored. The procedure is repeated until all items have been tested. To overcome scale indeterminacy, we imposed a linear restriction on the parameters of the reference group and allowed freely-estimated mean and variance parameter estimates in the focal group(s). This model configuration ensured that item parameter estimates could be compared between groups without containing latent distribution characteristics.

Differences in the item parameters a_i and b_i were tested jointly. Ethnic groups were compared overall (with Dutch arbitrarily defined as the reference group) and pairwise to understand which groups would be specifically affected by DIF. *P*-values were adjusted for multiple comparisons using the Bonferroni method. Additionally, the discrimination and difficulty parameters of the IRT model were estimated for each ethnic group using the multipleGroup() function in the "mirt" package, while the probability of a correct response for each item was also plotted across values of the latent trait (i.e. item characteristic curve) per ethnic group.

Criterion validity

We calculated a weighted-sum score of the latent variable as an *a posteriori* expectation for each sum score using the fscores() function in the "mirt" package. We regressed the weighted-sum score of antibiotic knowledge on answers to the questions related to the three antibiotic use behaviors using linear regression in separate models. We compared each response level to the "never" category as reference, which was assumed to be in

line with more appropriate antibiotic behaviors and hence associated with higher antibiotic knowledge. We calculated *p*-values based on a Wald χ^2 test.

RESULTS

Study population

Of the 23,942 participants who filled in the questionnaire, 586 were excluded as they belonged to ethnic groups with too few participants for meaningful analysis (i.e. Javanese Surinamese origin, *n*=250; other/unknown Surinamese origin, *n*=286; and other/unknown origin, *n*=50). In total, 23,356 participants were analyzed and belonged to the following ethnic groups: Dutch (*n*=4,641), South-Asian Surinamese (*n*=3,369), African Surinamese (*n*=4,458), Ghanaian (*n*=2,484), Turkish (*n*=4,067), and Moroccan (*n*=4,337). Characteristics of the study population have been described in a previous study (13).

Item response theory model and diagnostics

The distribution of response answers for the 3 items included in the final IRT model is described in Table 1. The most correctly answered item was Q2 on antibiotic use for influenza (80.6%), followed by Q4 for fever (77.9%) and Q3 for sore throat (71.7%). As also shown in Table 1, both the discrimination and difficulty parameters were highest for Q2, followed by Q4 and Q3.

Table 1. Distribution of responses to antibiotic knowledge questions and item response theory (IRT) model parameters in the HELIUS study (N=23,356), Amsterdam, the Netherlands (2011-2015)

Item	Response distribution		IRT parametersa	
	n	%	a	b
Item Q2: Influenza			3.161	3.110
Yes	3,865	16.5		
No (correct)	18,815	80.6		
Did not answer this question[b]	676	2.9		
Item Q3: Sore throat			1.750	1.399
Yes	5,941	25.4		
No (correct)	16,739	71.7		
Did not answer this question[b]	676	2.9		
Item Q4: Fever			2.323	2.231
Yes	4,405	18.9		
No (correct)	18,206	77.9		
Did not answer this question[b]	745	3.2		

[a]Item discrimination is represented with "a" and item difficulty with "b."
[b]Considered as "incorrect" in analysis.

Table 2. Assessing the item response theory model fit to data from the HELIUS study

Aspect assessed	Summary or statistic
Local dependence[a]	
Signed χ^2	Q2 versus Q3 (p=0.41), Q2 versus Q4 (p=0.37), Q3 versus Q4 (p=0.65)
Cramer's V	Q2 versus Q3 (0.005), Q2 versus Q4 (0.006), Q3 versus Q4 (0.003)
Goodness-of-fit[b]	
G2	103.9 (p<0.0001)
$RMSEA_2$	0.066

[a]Violation of local independence were tested using Signed χ^2 and Cramer's V statistics.
[b]Goodness-of-fit statistics include the G2 test (with 1 degree of freedom) and root-square mean of error approximation ($RMSEA_2$).

The statistics testing for violation of the local independence assumption are given in Table 2. The signed χ^2 test was not significant and Cramer's V was low for all pairwise comparisons between items, implying that local independence was held. The distribution of all possible response patterns and their expected totals are listed in Supplementary Table 4. All response patterns with only one correct response were much less common than expected. The G2 statistic showed significant departures from predicted response patterns (Table 2). Nevertheless, the $RMSEA_2$ indicated adequate model fit (Table 2).

DIF between ethnic groups

Overall evidence of DIF was observed for all three items (Table 3). DIF was also present in many of the pairwise comparisons between ethnic groups for all three items.

Given the evidence of DIF, we examined the distribution of response answers (Supplementary Table 5) and IRT model parameter estimates across ethnic groups (Supplementary Table 6). The percentage of individuals with correct responses on items varied across ethnic groups. Most individuals were able to correctly answer the item Q2 on antibiotic use for influenza (range: 68% in Ghanaian and 92% in Dutch), Q4 for fever (range: 71% in Ghanaian and 84% in Dutch), and Q3 for sore throat (range: 63% in Turkish and 84% in Dutch). The lowest item discrimination and item difficulty parameters were observed for Q3 (range: 1.488 in South-Asian Surinamese and 2.119 in Ghanaian; range: 0.856 in Turkish and 2.420 in Dutch; respectively), whereas the highest of these parameters were observed for Q2 (range: 2.020 in Turkish and 5.653 in South-Asian Surinamese; range: 1.986 in Turkish and 4.805 in South-Asian Surinamese; respectively). The item characteristic curve is given across ethnic groups in Figure 1.

Table 3. Differential item functioning between ethnic groups

Item	Overall test[a]	Pairwise comparisons between ethnic groups[b]
Item Q2: Influenza	p<0.0001	Dutch vs. South-Asian Surinamese (p<0.0001); Dutch vs. African Surinamese (p=0.0005); Dutch vs. Moroccan (p<0.0001); South-Asian Surinamese vs. Turkish (p<0.0001); African Surinamese vs. Ghanaian (p<0.0001); African Surinamese vs. Turkish (p=0.01); Ghanaian vs. Turkish (p<0.0001); Turkish vs. Moroccan (p<0.0001)
Item Q3: Sore throat	p<0.0001	Dutch vs. South-Asian Surinamese (p<0.0001); Dutch vs. African Surinamese (p<0.0001); Dutch vs. Turkish (p=0.0009); Dutch vs. Moroccan (p<0.0001); South-Asian Surinamese vs. Turkish (p<0.0001); African Surinamese vs. Ghanaian (p<0.0001); African Surinamese vs. Turkish (p=0.005); Ghanaian vs. Turkish (p=0.0001); Ghanaian vs. Moroccan (p=0.006); Turkish vs. Moroccan (p<0.0001)
Item Q4: Fever	p<0.0001	Dutch vs. South-Asian Surinamese (p<0.0001); Dutch vs. African Surinamese (p<0.0001); Dutch vs. Turkish (p<0.0001); Dutch vs. Moroccan (p<0.0001); African Surinamese vs. Ghanaian (p<0.0001); Ghanaian vs. Turkish (p=0.005); Ghanaian vs. Moroccan (p=0.001); Turkish vs. Moroccan (p=0.0002)

Differential item functioning was assessed with an auxiliary test approach (Methods, statistical analysis). *P*-values are calculated from a log-likelihood ratio test. Only results where p<0.05 are presented.
[a]Test comparing all ethnic groups as a focal group and the Dutch ethnic group as the reference group.
[b]Test between all pairwise comparisons between ethnic groups. *P*-values are adjusted using the Bonferroni method.

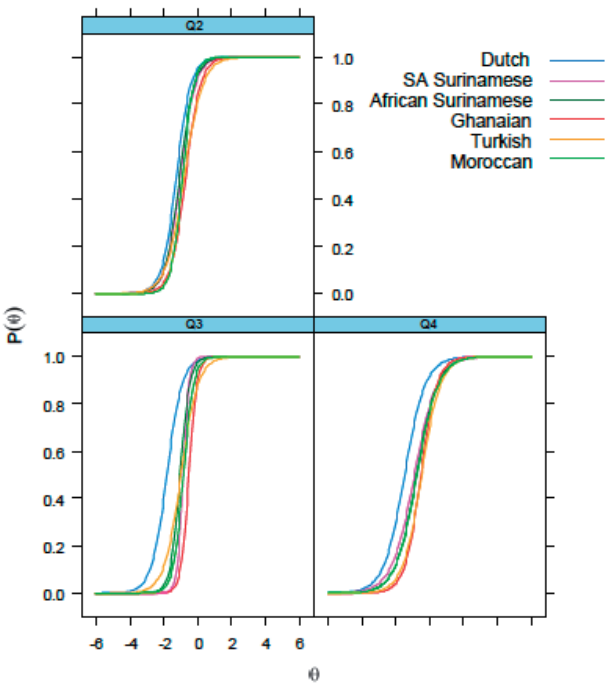

Figure 1. Item characteristic curve on knowledge of antibiotic use in the HELIUS study of Dutch (N=4,594), South-Asian (SA), Surinamese (N=3,267), African Surinamese (N=4,259), Ghanaian (N=2,339), Turkish (N=3,864) and Moroccan (N=3,992) origin.
Legend: Item characteristic curves depict the probability of a correct response, P(θ), in function of the latent trait, θ, which in this case represents antibiotic knowledge. Curves are stratified on ethnic groups. Items Q2, Q3, and Q4 correspond to those listed in Table 1.

Criterion-validity of antibiotic use knowledge with use behavior

Overall, 79.7% of participants never asked for antibiotics from a GP, compared to 17.0% and 1.3% who regularly and occasionally asked, respectively. 2.0% of participants did not respond to this question. Lower weighted-sum scores of antibiotic knowledge were found in those who both regularly and occasionally asked for antibiotics compared to never asking (Table 4). Furthermore, 87.2% always finished their antibiotic treatment, compared to 10.3% and 1.1% who regularly and occasionally finished (1.3% did not respond), and 96.8% never saved antibiotic treatment for later use, compared to 1.5% and 0.2% who regularly and occasionally saved (1.5% did not respond). Lower weighted-sum scores were found in those who both regularly and occasionally finished or saved antibiotics compared to always finishing or never saving antibiotics, respectively (Table 4). The association between antibiotic use behaviors and weighted-sum score was consistent across ethnic group (Supplementary Table 7), yet was slightly weaker in the Dutch group.

Table 4. Criterion-validity of antibiotic knowledge with respect to antibiotic use behaviors in the HELIUS study

Criterion	Linear regression of weighted-sum score[a]	
	Δ[b] (SE)	***p***
Ever asked for antibiotics from GP		
No, never	Ref	
Yes, regularly	-0.284 (0.042)	<0.001
Yes, occasionally	-0.193 (0.013)	<0.001
Missing	-0.330 (0.034)	<0.001
Did not finish treatment		
No, never	Ref	
Yes, regularly	-0.270 (0.046)	<0.001
Yes, occasionally	-0.132 (0.016)	<0.001
Missing	-0.613 (0.042)	<0.001
Saved antibiotic treatment		
Never	Ref	
Yes, regularly	-0.268 (0.110)	0.015
Yes, occasionally	-0.276 (0.039)	<0.001
Missing	-0.580 (0.040)	<0.001

[a]The weighted-sum score was obtained using the fscores() function in the "mirt" package.
[b]This parameter represents the mean difference between the given response category and reference group (Ref).

DISCUSSION

In this large, population-based, cross-sectional study, we studied the use of a short questionnaire to measure antibiotic knowledge across the most populous ethnic groups residing in Amsterdam, the Netherlands. We were able to show that antibiotic knowledge, as determined by three questions on appropriate antibiotic use, can be expressed as a latent trait with an IRT model showing adequate fit. Furthermore, higher weighted-sum scores of correct responses were associated with other behaviors that suggest adequate knowledge on antibiotic use. Our study provides psychometric insight on a rarely studied concept (22-24) and could be helpful for large-scale studies.

We acknowledge that the three-item questionnaire was relatively short compared to other inventories, namely those studying knowledge on cardiovascular disease (25), knowledge on diabetes mellitus (26), and mental illness literacy (27). The short length does make it easy to incorporate in large studies where individuals are asked to fill in lengthy questionnaires related to multiple disease outcomes. As this was the case in the HELIUS study, we explicitly asked a limited number of questions, two of which were poorly fit to the latent trait and ultimately had to be excluded in further analyses. It could be that a larger set of items would be able to capture antibiotic knowledge more robustly. Nevertheless, these larger inventories run the risk of including other dimensions related to antibiotic knowledge, thereby violating the unidimensionality intended with our questionnaire. Further study and validation of more extensive questionnaires is strongly suggested.

Interestingly, the items retained in the final IRT model are commonly used to describe symptoms resulting from both the common cold and influenza (28), whereas the excluded items on bronchitis and pneumonia are more reflective of a specific illness. Antibiotic knowledge, as measured in this study, seems driven by perceptions of needing antibiotic therapy for these symptoms, or rather not needing antibiotic therapy considering that the correct answer to all three items was “no”. Given the fact that items related to specific illnesses had poor fit and mostly poor discrimination (Supplementary Table 3), there would appear to be a large amount of confusion overall on when to treat these diseases with antibiotics.

By asking questions on behaviors related to antibiotic use, we were able to assess criterion-validity using the weighted-sum score of antibiotic knowledge. We observed that participants either regularly or occasionally asking for antibiotics from the GP, regularly or occasionally not finishing antibiotic treatment, and regularly or occasionally saving antibiotics all had lower weighted-sum scores of antibiotic knowledge when compared

to those with more appropriate antibiotic use behaviors. However, the questions on asking antibiotics from the GP and finishing antibiotic treatment were asked to all participants regardless of previous exposure to antibiotic treatment. It is then difficult to determine if experience with antibiotic treatment is associated with higher antibiotic knowledge. It is also noteworthy that individuals who did not respond to questions on antibiotic use behaviors had much lower weighted-sum scores compared to those with more appropriate antibiotic use behaviors, suggesting that missingness related to these questions may reflect lower antibiotic knowledge. Taken together, there is evidence for criterion validity in our study.

DIF was present for many of the items–implying that individuals from different ethnic groups had different probabilities of responding correctly to an item despite having the same latent trait. It should be noted that the methods used to identify DIF are susceptible to type 1 error with large sample sizes (21), which might explain the pervasiveness of DIF. Furthermore, when the analysis on criterion validity, which used the weighted-sum score from the IRT model, was stratified by ethnic groups, the absolute differences were comparable across ethnicities and conclusions with respect to significance were for the most part maintained. In this regard, the downstream consequences of DIF could be considered ignorable. Perhaps the observed differences in percentages of correctly answered questions between ethnic groups might not result from variation in antibiotic knowledge, but rather antibiotic use practices in the countries of origin. A higher defined daily dose/1000 inhabitants per day has been observed in countries such as Turkey (at 38.2), compared to the Netherlands (at 9.8) (29), which indicates more lenient perception practices in countries outside the Netherlands. These practices could influence the perception for needing antibiotics, yet should be clarified.

Strengths of the study include large numbers of participants from major ethnic groups living in the same city, with a wide range of socioeconomic levels. Translated questionnaires and ethnically-matched interviewers were used to enhance cultural comparability. Furthermore, few studies have made use of IRT modeling to assess antibiotic knowledge (30-32) and to the best of our knowledge, this is the first to examine differences in measuring antibiotic knowledge between ethnic groups. Nevertheless, some limitations need to be addressed. First, this study was conducted in a concentrated geographical region and external validation of our results is needed in other settings. Second, given the few questionnaire items, we were unable to test for multidimensionality. The items used herein reflected the use of antibiotics specific to given symptoms or diseases and thus, these items should be theoretically unidimensional. Further research should explore other dimensions, such as awareness and attitudes, and whether they relate to knowledge of use. Finally, few individuals regularly asked their GP for antibiotics or

regularly finished antibiotics, hence statistical power to establish criterion-validity for these levels, particularly within ethnic groups, was likely insufficient. Furthermore, the questions used to establish criterion-validity might not have theoretically represented the construct of antibiotic knowledge based on indicated use, but rather could reflect an independent construct of antibiotic knowledge altogether.

In conclusion, this 3-item questionnaire could be used to determine antibiotic knowledge across diverse populations. There appeared to be differences in item functioning across ethnic groups, but analysis using outputs from the IRT model would suggest that this DIF can be ignored. This questionnaire would be ideal for large-scale studies in which measuring the effect of antibiotic knowledge is part of a broader scope of research objectives. Given that the questions evaluated in this study only reflect appropriate use of antibiotics as it relates to specific symptoms, other inventories will need to be developed to assess different dimensions of antibiotic knowledge.

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Conflicts of interest

The authors declare that they have no competing interests.

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Supplementary Table 1. Correct responses to the questions related to antibiotic knowledge asked in the HELIUS study

Item	Question	Correct response
Q1	Do you think a physician would find it necessary to prescribe antibiotics for bronchitis?	No
Q2	Do you think a physician would find it necessary to prescribe antibiotics for influenza?	No
Q3	Do you think a physician would find it necessary to prescribe antibiotics for sore throat?	No
Q4	Do you think a physician would find it necessary to prescribe antibiotics for fever?	No
Q5	Do you think a physician would find it necessary to prescribe antibiotics for pneumonia?	Yes

Supplementary Table 2. Questions related to antibiotic use behaviors asked in the HELIUS study

Item	Question	Response levels
Ever asked for antibiotics from GP	Have you ever asked the doctor to prescribe antibiotics?	No, never Yes, regularly Yes, occasionally Missing
Did not finish treatment	Have you ever not finished an antibiotic prescription?	No, never Yes, regularly Yes, occasionally Missing
Saved antibiotic treatment	Have you ever saved an antibiotic prescription that you did not finish for another time?	No, never[a] Yes, regularly Yes, occasionally Missing

[a]This question depended on the response to "did not finish treatment". If a participant reported "No, never" to the question, "Have you ever not finished an antibiotic prescription?", the response to saving antibiotic treatment was imputed as "No, never."

Supplementary Table 3. Item response theory (IRT) model parameters for antibiotic knowledge in the HELIUS study, exploring configurations of included items

Item	Response distribution		IRT parametersa					
			5-item model		4-item model (excluding Q5)		3-item model (excluding Q5 and Q1)	
	n	%	a	b	a	b	a	b
Item Q1: Bronchitis			0.616	0.225	0.524	0.220	-	-
Yes	9,746	41.7						
No (correct)	12,883	55.2						
Did not answer this question[b]	727	3.1						
Item Q2: Influenza			2.731	2.785	2.960	2.952	3.161	3.110
Yes	3,865	16.5						
No (correct)	18,815	80.6						
Did not answer this question[b]	676	2.9						
Item Q3: Sore throat			1.805	1.422	1.782	1.410	1.750	1.399
Yes	5,941	25.4						
No (correct)	16,739	71.7						
Did not answer this question[b]	676	2.9						
Item Q4: Fever			2.486	2.331	2.391	2.268	2.323	2.231
Yes	4,405	18.9						
No (correct)	18,206	77.9						
Did not answer this question[b]	745	3.2						
Item Q5: Pneumonia			-0.305	1.103	-	-	-	-
Yes (correct)	17,436	74.7						
No	5,396	23.1						
Did not answer this question[b]	524	2.2						

[a]Item discrimination is represented with "a" and item difficulty with "b."
[b]Considered as "incorrect" in analysis.

Supplementary Table 4. Frequencies for the item response theory model fitted to the HELIUS data

Response pattern	Observed frequency	Expected	Residual
000	2,165	2,024.771	3.116
001	781	915.013	-4.430
010	609	741.850	-4.878
011	986	880.403	3.559
100	872	1,012.808	-4.424
101	2,799	2,677.064	2.357
110	1,504	1,386.451	3.157
111	13,640	13,717.640	-0.663

Response patterns are depicted as correct (1) or incorrect (0) answers to items Q2-Q4 in consecutive order. Observed frequencies are provided along with marginal expected frequencies for each response pattern.

Supplementary Table 5. Distribution of responses to questions related to antibiotic knowledge in the HELIUS study (N=23,356), Amsterdam, the Netherlands, stratified by ethnicity

Antibiotic knowledge questions	Ethnicity*											
	Dutch		South-Asian Surinamese		African Surinamese		Ghanaian		Turkish		Moroccan	
	(N=4,641)		(N=3,369)		(N=4,458)		(N=2,484)		(N=4,067)		(N=4,337)	
	n	%	n	%	n	%	n	%	n	%	n	%
Item Q1: Bronchitis												
Yes	2,278	49	1,506	45	1,983	44	984	40	1,357	33	1,638	38
No (correct)	2,332	50	1,803	53	2,344	53	1,391	56	2,582	64	2,431	56
Did not answer this question	31	0.7	60	2	131	3	109	4	128	3	268	6
Item Q2: Influenza												
Yes	330	7	634	19	654	15	695	28	821	20	731	17
No (correct)	4,279	92	2,672	79	3,671	82	1,698	68	3,142	77	3,353	77
Did not answer this question	32	0.7	63	2	133	3	91	4	104	3	253	6
Item Q3: Sore throat												
Yes	682	15	867	26	1,177	26	753	30	1,376	34	1,086	25
No (correct)	3,923	84	2,436	72	3,149	71	1,629	66	2,584	63	3,018	70
Did not answer this question	36	0.8	66	2	132	3	102	4	107	3	233	5
Item Q4: Fever												
Yes	696	15	628	19	739	17	618	25	1,027	25	697	16
No (correct)	3,911	84	2,665	79	3,567	80	1,771	71	2,917	72	3,375	78
Did not answer this question	34	0.7	76	2	152	3	95	4	123	3	265	6

Supplementary Table 5. Distribution of responses to questions related to antibiotic knowledge in the HELIUS study (N=23,356), Amsterdam, the Netherlands, stratified by ethnicity *(continued)*

Antibiotic knowledge questions	Ethnicity*											
	Dutch		South-Asian Surinamese		African Surinamese		Ghanaian		Turkish		Moroccan	
	(N=4,641)		(N=3,369)		(N=4,458)		(N=2,484)		(N=4,067)		(N=4,337)	
Item Q5: Pneumonia												
Yes (correct)	4,226	91	2,565	76	3,338	75	1,400	56	2,881	71	3,026	70
No	391	8	754	22	1,029	23	993	40	1,091	27	1,138	26
Did not answer this question	24	0.5	50	1	91	2	91	4	95	2	173	4

Participants with a Javanese (n=250), Surinamese 'other' (n=286) or an unknown or 'other' (n=50) ethnicity were excluded.

Supplementary Table 6. Item response theory (IRT) model parameters for antibiotic knowledge in the HELIUS study (N=23,356), Amsterdam, the Netherlands, stratified by ethnic group

Item	IRT parameters[a]											
	Dutch		South-Asian Surinamese		African Surinamese		Ghanaian		Turkish		Moroccan	
	a	b	a	b	a	b	a	b	a	b	a	b
Item Q2: Influenza	2.212	4.027	5.653	4.805	3.512	3.638	4.265	2.194	2.020	1.986	3.349	2.814
Item Q3: Sore throat	1.637	2.420	1.488	1.334	1.633	1.278	2.119	1.092	1.798	0.856	1.599	1.192
Item Q4: Fever	2.371	2.963	2.287	2.323	2.327	2.443	2.326	1.628	1.851	1.448	3.031	2.668

[a]Item discrimination is represented with “a” and item difficulty with “b.” Parameter estimates were obtained using the multipleGroup() function in the “mirt” package in R.

Supplementary Table 7. Criterion-validity of antibiotic knowledge[a] with respect to antibiotic use behaviors in the HELIUS study, stratified by ethnic group

	Dutch		South-Asian Surinamese		African Surinamese		Ghanaian		Turkish		Moroccan	
	Δ[b] (SE)	*p*	Δ[b] (SE)	*p*	Δ[b] (SE)	*p*	Δ[b] (SE)	*p*	Δ[b] (SE)	*p*	Δ[b] (SE)	*p*
Ever asked GP for antibiotics												
No, never	Ref		Ref		Ref		Ref		Ref		Ref	
Yes, regularly	-0.195 (0.089)	0.03	-0.353 (0.118)	0.003	-0.193 (0.134)	0.15	-0.024 (0.127)	0.85	-0.489 (0.087)	<0.001	-0.152 (0.084)	0.07
Yes, occasionally	-0.068 (0.022)	0.002	-0.223 (0.034)	<0.001	-0.148 (0.030)	<0.001	-0.254 (0.044)	<0.001	-0.262 (0.029)	<0.001	-0.193 (0.031)	<0.001
Missing	-0.314 (0.106)	0.003	-0.278 (0.115)	0.02	-0.342 (0.080)	<0.001	-0.477 (0.098)	<0.001	-0.191 (0.083)	0.02	-0.210 (0.062)	<0.001
Did not finish treatment												
No, never	Ref		Ref		Ref		Ref		Ref		Ref	
Yes, regularly	-0.557 (0.254)	0.03	-0.113 (0.104)	0.28	-0.372 (0.105)	<0.001	-0.303 (0.116)	0.009	-0.176 (0.107)	0.102	-0.059 (0.106)	0.58
Yes, occasionally	-0.050 (0.032)	0.12	-0.169 (0.041)	<0.001	-0.114 (0.032)	<0.001	-0.127 (0.064)	0.046	-0.133 (0.037)	<0.001	-0.116 (0.036)	0.001
Missing	-0.413 (0.114)	<0.001	-0.383 (0.118)	0.001	-0.543 (0.095)	<0.001	-0.617 (0.100)	<0.001	-0.508 (0.102)	<0.001	-0.736 (0.094)	<0.001
Saved antibiotic treatment												
Never	Ref		Ref		Ref		Ref		Ref		Ref	
Yes, regularly	0.314 (0.401)	0.43	-0.026 (0.278)	0.93	-0.257 (0.239)	0.28	-0.470 (0.368)	0.20	-0.232 (0.210)	0.27	-0.272 (0.267)	0.31
Yes, occasionally	-0.068 (0.091)	0.46	-0.314 (0.108)	0.004	-0.172 (0.083)	0.038	-0.324 (0.103)	0.002	-0.182 (0.090)	0.04	-0.360 (0.101)	<0.001
Missing	-0.465 (0.112)	<0.001	-0.355 (0.112)	0.001	-0.529 (0.093)	<0.001	-0.538 (0.094)	<0.001	-0.439 (0.096)	<0.001	-0.719 (0.089)	<0.001

[a]Defined as the weighted-sum score was obtained using the fscores() function in the "mirt" package.

[b]This parameter represents the mean difference between the given response category and reference group (ref).

4

Nasal carriage of methicillin-resistant *Staphylococcus aureus* (MRSA) among undocumented migrants and uninsured legal residents in Amsterdam, the Netherlands: a cross-sectional study.

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ABSTRACT

Background

Nasal carriage of methicillin-resistant *Staphylococcus aureus* (MRSA) is associated with an increased risk of infection. Colonization with MRSA is observed in <1% of the general Dutch population. Increased risk for MRSA carriage is known to occur in several key groups, one of which is asylum seekers. However, little is known about MRSA carriage among undocumented migrants and uninsured legal residents. This study aimed to determine the prevalence of nasal MRSA carriage among these groups in Amsterdam, the Netherlands.

Methods

In this cross-sectional study, between October 2018 and October 2019, undocumented migrants and uninsured legal residents aged 18 years or older who were able to understand one of the study languages were recruited at an NGO health care facility in Amsterdam, the Netherlands, for general practitioner (GP) consultations. Participants were asked questions on demographics, migration history, antibiotic use and other possible risk factors for MRSA carriage and were screened for nasal MRSA carriage by selective culturing e-swabs. Characteristics of MRSA-negative and MRSA-positive participants were compared using univariable logistic regression analysis with Firth's correction.

Results

Of the 3,822 eligible patients, 760 were screened for nasal MRSA carriage (19.9%). Of the 760 participants, over half were male (58%; 442/760) and originated mainly from Africa (35%; 267/760), Asia (30%; 229/760) and North or South America (30%; 227/760). In total, 705/760 participants (93%) were undocumented migrants and 55/760 (7%) were uninsured legal residents of Amsterdam. The overall prevalence of nasal MRSA carriage was 2.0% (15/760) (95%CI 1.1% to 3.2%), with no difference between undocumented migrants (14/705) (2.0%, 95%CI 1.1% to 3.3%) and uninsured legal residents (1/55) (1.8%, 95%CI 0.1% to 9.7%). Genotyping showed no clustering of the 15 isolates. MRSA carriage was not associated with sociodemographic, migration history or other possible risk factors. Nevertheless, this study had limited power to detect significant determinants. Three participants (3/15; 20%) harbored Panton-Valentine leukocidin (PVL)-positive isolates.

Conclusions

Even though our study population of undocumented migrants and uninsured legal residents had a higher prevalence of nasal MRSA carriage compared to the general Dutch population, the prevalence was relatively low compared to acknowledged other high-risk groups.

BACKGROUND

Staphylococcus aureus is a commensal bacterium, but frequently causes clinically important nosocomial or community-acquired infections(1). During infection with methicillin-resistant *S. aureus* (MRSA), treatment options are limited. Even though the number of MRSA infections is still relatively low in many Western and Northern European countries, approximately 150,000 infections of MRSA occur each year in the European Union, accounting for an estimated 7,000 deaths annually(2).

Nasal carriage of *S. aureus* in general is associated with an increased risk of MRSA infection(3, 4). Consequently, the Netherlands has taken an aggressive approach to prevent the spread of MRSA in those with MRSA carriage. As part of the Dutch MRSA search and destroy policy, all known MRSA carriers, as well as their household and in-hospital contacts, and all patients who have been admitted to foreign hospitals for more than 24 hours in the previous two months are isolated at hospital admission(5, 6). Isolation is prolonged until screening cultures for MRSA are negative or MRSA carriage is eradicated. Coupled with the general reluctance of prescribing antibiotics among Dutch physicians (7), the prevalence of MRSA carriage is <0.2% of new hospital admissions in the Netherlands(8, 9).

MRSA carriage is observed in <1% of the general Dutch population (5), yet certain groups are known to have a higher prevalence. For instance, a recent study from the Netherlands has shown that 10% of asylum seekers were carriers of MRSA(10). This prevalence falls in line with a recent systematic review and meta-analysis among migrants in Europe in which a pooled 8% prevalence of MRSA carriage was estimated(11). Nevertheless, other conducted studies in similar settings have reported widely varying prevalences, with a Norwegian study reporting MRSA carriage in 0.74% of asylum seekers (12) and one Finnish study reporting MRSA carriage in 21% of asylum seekers and refugees(13). Part of this variation could be due to the variation in the countries of origin of migrants included in these studies.

Undocumented migrants (including rejected asylum seekers, migrants with expired visa and 'directly undocumented migrants', i.e. those who bypassed the asylum procedure) and uninsured legal residents are thought to represent a considerable fraction of migrants residing in the Netherlands, yet the exact proportion is unknown. Multiple international studies on immigration and the impact of immigration policies have shown an association between undocumented migration and poorer health outcomes(14-16). In particular, the prevalence of infectious diseases (e.g. human immunodeficiency virus (HIV), hepatitis B virus (HBV), hepatitis C virus (HCV) and tuberculosis (TB)) tends to

be higher among homeless individuals and undocumented migrants(17-21). However, little is known about the proportion of MRSA-carriers among undocumented migrants and uninsured legal residents in the Netherlands. These individuals are known to live in more difficult socioeconomic situations (e.g. crowded living conditions) (22, 23), which could make them more vulnerable to inadequate care and possibly at higher risk for MRSA carriage. To the best of our knowledge, the prevalence of MRSA carriage in this population has not yet been studied. This study aimed to determine the prevalence of nasal MRSA carriage among undocumented migrants and uninsured legal residents in Amsterdam, the Netherlands.

METHODS

Study design, setting and population

A cross-sectional study was designed to evaluate the prevalence of HBV, HCV, HIV and MRSA carriage in individuals seeking care at Kruispost, a low-threshold care facility for undocumented migrants and (Dutch) homeless and uninsured individuals. A sample of 1000 participants was intended to be recruited from patients visiting Kruispost for an appointment with a general practitioner (GP) during a one-year period. We based sample size on the capacity of Kruispost to recruit participants in a 12-month time span (i.e. convenience sample). Between October 2018 and October 2019, visitors aged 18 years or older who were able to understand one of the study languages (Dutch, English, French, Spanish, Arabic and Portuguese) were invited to participate.

Prior to June 20, 2019, we excluded patients originating from countries within the European Union (EU) and/or European Economic Area (EEA) who did not possess a citizen service number (CSN), since treatment could not be reimbursed by the central administration office (CAK)-regulation during that time. The CAK is a public service provider that carries out regulations and translates legislations on behalf of the government. CAK-regulation reimburses medical treatment for uninsurable individuals under specific circumstances. As of June 20, 2019, this reimbursement regulation was extended to include individuals originating from countries within the EU or EEA without a CSN and thus after this date, these patients were also invited to participate.

In this report, we provide results on the MRSA screening component of the study. Therefore, only participants with an MRSA screening result were included in the analysis.

Study procedures

After GP consultation, patients were invited to participate in the study. Eligible patients were provided with study information and if willing to participate, gave oral informed consent. Patients who declined to participate were asked to complete a short questionnaire on demographics and reason(s) for non-participation. All participants were offered an incentive (a ticket for public transportation, socks, toothbrush, shampoo or disinfecting hand gel).

Participants completed two questionnaires: the first filled out together with a research associate (including only information on risk factors for HBV/HCV infection to determine eligibility for HBV/HCV screening) and the second self-administered (including all other information). Information obtained from the questionnaires included sociodemographic variables (age, sex, country of birth, educational level), migration history (year of leaving country of origin, year of arrival in the Netherlands, way of entering the Netherlands, housing situation, the number of housemates they currently live with), antibiotic use (current use and use in the past six months) and other variables on potential risk factors (whether or not they had been abroad for more than 24 hours in the past six months, whether they have ever been admitted or treated in a foreign hospital, had surgery abroad, had a blood transfusion, had paid or had been paid for sex, and injected drugs).

On the day of informed consent, a nasal swab was taken by a research assistant to be screened for MRSA. All positive MRSA diagnoses were added to the electronic health record dossier (EHR) of Kruispost participants to inform healthcare providers in the event of (future) referral to secondary care. Since treatment of MRSA carriage is not indicated outside of hospital settings, we decided not to inform patients of their MRSA status. Participants were informed, however, that their MRSA status would be added to their EHR in case of a positive test.

Laboratory detection

Collected e-swabs (Copan, Brescia, Italy) were sent to the laboratory of the Public Health Service of Amsterdam by mail at the end of the day of sample collection. Transport time was 24-48 hours by mail. The detection of MRSA was done according to the NVMM (Dutch Society for Medical Microbiology) guidelines for laboratory detection of highly-resistant microorganisms(24). In brief, culture for MRSA was done by overnight enrichment in broth containing 6% NaCl, followed by subculture on selective chromogenic plates (CHROMID MRSA, Biomerieux, Marcy-l'Étoile, France), which were read after 24 and 48 hours. All cultures were done at 36°C. *S. aureus* strains were identified by Maldi-TOF MS (Bruker, Massachusetts, United States of America). MRSA phenotype was confirmed by oxacillin E-test (BioMerieux, Marcy l'Étoile, France) and a PBP2A agglutination test

(Alere, Massachusetts, United States of America). Presence of the *mecA* gene was confirmed at the National Institute of Public Health and the Environment (RIVM) by PCR(25). Isolates were assessed for the presence of Panton-Valentine leukocidin (PVL) gene by PCR(26), which is mainly observed in community-associated MRSA(27) and, in general, is a virulence marker associated with more severe skin and soft tissue infections. Typing of strains isolated in this study was done by Multi-Locus Variable Number Tandem Repeat Analysis (MLVA) as is done with all MRSA strains isolated in the Netherlands in the nationwide MRSA surveillance (28).

Statistical analyses

Sociodemographics, questions on foreign treatments and antibiotic use were presented by MRSA status. Years since leaving the country of origin and years since arrival in the Netherlands were calculated. Comparisons between groups were made using Fisher exact test for categorical data and by Mann-Whitney U test for continuous data. Prevalence of MRSA carriage and its corresponding Clopper-Pearson 95% confidence interval (CI) were calculated. Odds ratios (OR) comparing odds for MRSA carriage across levels of determinants, along with their 95%CI, were assessed using univariable logistic regression with Firth's correction. The small number of MRSA-positive samples in our study precluded any multivariable analysis. The significance level was set at $p<0.05$. All analyses were conducted with Stata 15.1 (StataCorp., College Station, Texas, USA).

RESULTS

Participants

In total, 4,017 patients visited Kruispost during the inclusion period. Of them, 89/4,017 (2%) were aged <18 years and 106/4,017 (3%) were unable to understand one of the six study languages. In total, 3,822/4,017 (95%) eligible patients remained. Of them, 1,376 (36%) were invited to participate, and 760 (19.9%) were screened for nasal MRSA carriage. (Figure 1). Supplementary table 1 compares the characteristics of those who did versus those who did not participate (restricted to non-participants completing the short questionnaire on demographics). Participants more often originated from Africa and Asia and left their country of origin less recently than non-participants. Furthermore, non-participants were more often European citizens compared to study participants. Supplementary table 2 shows the reasons for non-participation among patients who completed a short questionnaire on demographics (33% of total non-participants).

Of those who participated, the median age was 40 years (interquartile range (IQR) 31-50) and 58% (442/760) were men. 705/760 participants (93%) were undocumented migrants

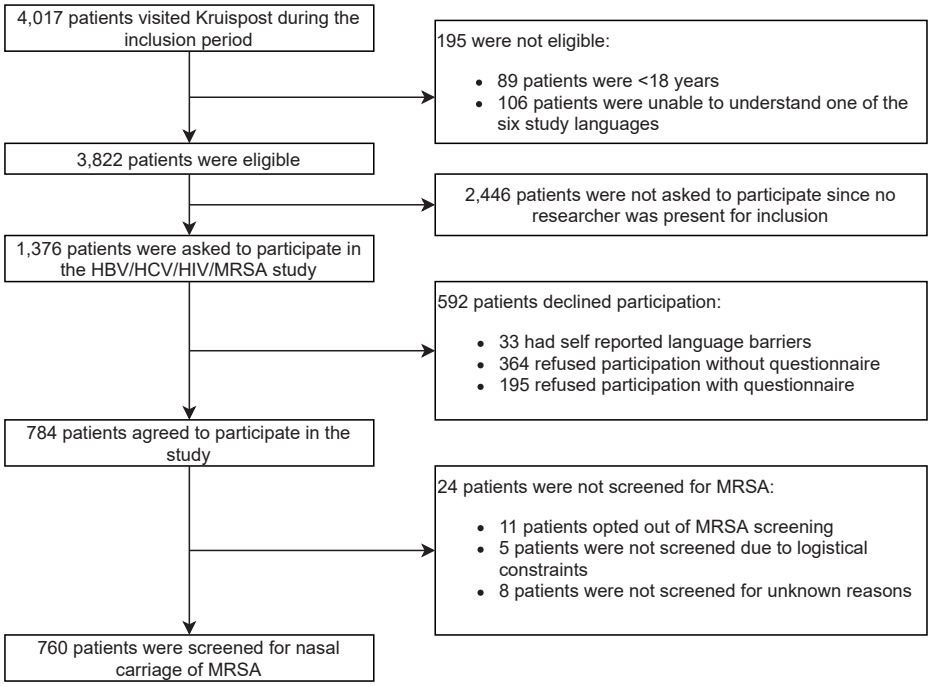

Figure 1. Recruitment strategy of MRSA screening offered to undocumented migrants attending Kruispost in Amsterdam, the Netherlands (N=4,017)
Abbreviations: HBV = Hepatitis B Virus; HCV = Hepatitis C Virus; HIV = Human Immunodeficiency Virus; MRSA = Methicillin-resistant *Staphylococcus aureus* `

and 55/760 participants (7%) were uninsured legal residents of Amsterdam. Participants originated mainly from Africa (35%; 267/760), Asia (30%; 229/760) and North or South America (30%; 227/760) and the majority completed secondary school (42%; 320/760) or higher education (36%; 274/760). The ways of entering the Netherlands were diverse across participants, but most indicated arriving on a (now expired) tourist, working or student visa (54%; 406/760), being a rejected asylum seeker (18%; 135/760), or illegally crossing borders (16%; 117/760). Five percent of participants (38/760) reported current use of antibiotics and 25% (186/760) being abroad for more than 24 hours in the past six months. Of participants, 36% (268/760) reported admission to a foreign hospital and 33% (254/760) had surgery abroad (Table 1).

Prevalence of and risk factors for nasal MRSA carriage

A total of 15 participants were MRSA-positive for nasal carriage, resulting in an overall prevalence of 2.0% (95%CI 1.1% to 3.2%). This prevalence was comparable between undocumented migrants (14/705) (2.0%, 95%CI 1.1% to 3.3%) and uninsured legal residents (1/55) (1.8%, 95%CI 0.1% to 9.7%). The median age of MRSA carriers was 35 years

Table 1. Characteristics of undocumented migrants and uninsured legal residents (N=760) and determinants of nasal MRSA carriage, in Amsterdam, the Netherlands, October 2018 - October 2019 (univariable logistic regression analyses with Firth's correction)

	Total (N=760)		MRSA-negative participants (N=745)		MRSA-positive participants (N=15)		Univariable associations	
	n	%	n	%	n	%	OR	95%CI
Sociodemographic variables								
Sex								
Male	442	58%	431	58%	11	73%	Ref	
Female	317	42%	313	42%	4	27%	0.54	0.18-1.62
Other	1	0.1%	1	0.1%	0	0%	*	*
Age in years, median (IQR)	40	(31-50)	40	(31-50)	35	(32-48)		
Age, categorized								
<35 years	249	33%	243	33%	6	40%	Ref	
35-49 years	302	40%	295	40%	7	47%	0.95	0.33-2.76
50-64 years	183	24%	181	24%	2	13%	0.52	0.12-2.25
≥65 years	26	3%	26	3%	0	0%	0.71	0.04-12.90
Kruispost target population								
Undocumented migrant	705	93%	691	93%	14	93%	Ref	
Uninsured legal resident	55	7%	54	7%	1	7%	1.31	0.24-7.21
Region of birth								
Europe	36	5%	36	5%	0	0%	Ref	
Asia	229	30%	222	30%	7	47%	2.46	0.14-44.01

Table 1. Characteristics of undocumented migrants and uninsured legal residents (N=760) and determinants of nasal MRSA carriage, in Amsterdam, the Netherlands, October 2018 - October 2019 (univariable logistic regression analyses with Firth's correction) *(continued)*

	Total (N=760)		MRSA-negative participants (N=745)		MRSA-positive participants (N=15)		Univariable associations	
	n	%	n	%	n	%	OR	95%CI
Africa	267	35%	261	35%	6	40%	1.81	0.10-32.89
North/South America	227	30%	225	30%	2	13%	0.81	0.04-17.20
Educational level								
No school	33	4%	33	4%	0	0%	Ref	
Primary school	129	17%	125	17%	4	27%	2.40	0.13-45.74
Secondary school	320	42%	314	42%	6	40%	1.38	0.08-25.13
Higher education	274	36%	269	36%	5	33%	1.37	0.07-25.28
Migration history								
Year of leaving country of origin, median (IQR)	2011	(2003-2016)	2011	(2003-2016)	2016	(2009-2016)		
Year of leaving country of origin, categorized								
<2010	335	45%	330	45%	5	33%	Ref	
2010-2017	329	44%	321	44%	8	53%	1.59	0.54-4.69
≥2018	88	12%	86	12%	2	13%	1.74	0.38-7.89
Years since leaving country of origin, median (IQR)	8	(3-16)	8	(3-16)	3	(3-10)	0.98	0.93-1.04
Year of arrival in the Netherlands, median (IQR)	2013	(2006-2017)	2013	(2006-2017)	2016	(2012-2018)		
Year of arrival in the Netherlands								
<2010	279	37%	276	37%	3	20%	Ref	

Table 1. Characteristics of undocumented migrants and uninsured legal residents (N=760) and determinants of nasal MRSA carriage, in Amsterdam, the Netherlands, October 2018 - October 2019 (univariable logistic regression analyses with Firth's correction) *(continued)*

	Total (N=760)		MRSA-negative participants (N=745)		MRSA-positive participants (N=15)		Univariable associations	
	n	%	n	%	n	%	OR	95%CI
2010-2017	308	41%	300	41%	8	53%	2.23	0.64-7.84
≥2018	167	22%	163	22%	4	27%	2.17	0.53-8.91
Years since arrival in the Netherlands, median (IQR)	5	(2-13)	6	(2-13)	3	(1-7)	0.97	0.91-1.04
Way of entering the Netherlands								
Expired tourist/working/student visa	406	54%	398	54%	8	53%	Ref	
Rejected asylum seeker	135	18%	133	18%	2	13%	0.88	0.21-3.65
EU citizen	40	5%	39	5%	1	7%	1.78	0.30-10.41
Illegally crossing borders	117	16%	113	15%	4	27%	1.86	0.58-5.94
Legally/other visa**/work	37	5%	37	5%	0	0%	0.63	0.04-11.04
Other/unknown	18	2%	18	2%	0	0%	1.27	0.07-22.80
Housing situation (multiple answers possible)								
Lives in BBB facility	56	7%	55	7%	1	7%	1.29	0.23-7.07
Lives with friends/family	401	53%	394	53%	7	47%	0.79	0.29-2.12
Lives in illegal rent	169	22%	166	22%	3	20%	0.97	0.29-3.23
Lives in housing provided by charity	64	9%	63	8%	1	7%	1.11	0.20-6.09
Lives on the streets	60	8%	59	8%	1	7%	1.19	0.22-6.55
Lives in other housing#	51	7%	49	7%	2	13%	2.61	0.66-10.36

Table 1. Characteristics of undocumented migrants and uninsured legal residents (N=760) and determinants of nasal MRSA carriage, in Amsterdam, the Netherlands, October 2018 - October 2019 (univariable logistic regression analyses with Firth's correction) *(continued)*

	Total (N=760)		MRSA-negative participants (N=745)		MRSA-positive participants (N=15)		Univariable associations	
	n	%	n	%	n	%	OR	95%CI
Number of housemates								
No housemates	105	14%	103	14%	2	13%	Ref	
<3	335	45%	329	45%	6	40%	0.82	0.19-3.57
3-5	228	31%	223	30%	5	33%	1.02	0.22-4.63
≥6	79	11%	77	11%	2	13%	1.34	0.23-7.90
Antibiotic use								
Current antibiotic use	38	5%	36	5%	2	13%	3.55	0.88-14.25
Recent antibiotic use								
Current or <3 months ago	89	12%	87	12%	2	14%	Ref	
3-6 months ago	54	7%	53	7%	1	7%	0.98	0.13-7.64
>6 months ago or never used antibiotics	454	63%	447	63%	7	50%	0.59	0.14-2.50
Does not remember when last using antibiotics	125	17%	121	17%	4	29%	1.30	0.27-6.23
Other potential risk factors								
Has been abroad for >24 hours in the past 6 months	186	25%	183	25%	3	20%	0.85	0.26-2.82
Ever admitted/treated in a foreign hospital	268	36%	265	36%	3	20%	0.50	0.15-1.65
Ever had surgery abroad	254	33%	250	34%	4	27%	0.77	0.26-2.33

Table 1. Characteristics of undocumented migrants and uninsured legal residents (N=760) and determinants of nasal MRSA carriage, in Amsterdam, the Netherlands, October 2018 - October 2019 (univariable logistic regression analyses with Firth's correction) *(continued)*

	Total (N=760)		MRSA-negative participants (N=745)		MRSA-positive participants (N=15)		Univariable associations	
	n	%	n	%	n	%	OR	95%CI
Ever received a blood transfusion								
No/unknown	717	94%	703	94%	14	93%	Ref	
Yes	43	6%	42	6%	1	7%	1.71	0.31-9.46
Ever (been) paid for sex								
No, never	613	81%	600	81%	13	87%	Ref	
Yes, ever paid	123	16%	121	16%	2	13%	0.92	0.23-3.58
Yes, ever been paid	24	3%	24	3%	0	0%	0.91	0.05-15.72
Ever injected drugs	16	2%	15	2%	1	7%	4.88	0.84-28.19

* Insufficient estimation of OR and variance due to '0' count cell

** Includes family visa and Schengen visa

Includes legal rent, housing for asylum seekers, boats, employers, hotels, crisis care, winter care, campers and forest huts

Missing data: region of birth, n=1; education, n=4; year of leaving country of origin, n=8; year of arrival in the Netherlands, n=6; way of entering the Netherlands, n=7; number of housemates, n=13; current antibiotic use, n=10; recent antibiotic use, n=38; abroad in past six months, n=4; admitted to foreign hospital, n=7

Abbreviations: MRSA – methicillin-resistant *Staphylococcus aureus*; OR – odds ratio; CI – confidence interval; IQR – inter quartile range; EU – European Union; BBB – bed bath bread

(IQR 32-48) and 73% (11/15) of carriers was male. Table 1 shows the characteristics of MRSA carriers and non-carriers. As shown in Table 1, MRSA carriage was not associated with any sociodemographic variable, migration history or other possible risk factors.

MRSA genotyping

Of the 15 isolates from MRSA-positive participants (Table 2), 3 (3/15; 20%) were Panton-Valentine leukocidin (PVL)-positive. Fourteen different MLVA-types were detected. None of the participants had livestock-associated MRSA. Eleven patients (11/15; 73%) had MLVA-types that have never or rarely been found in both the Amsterdam region and nationwide. Four (4/15; 27%) participants had MLVA-types that have been regularly (more than 25 times) isolated in other persons outside the Amsterdam region in the

Table 2. Genetic characteristics of MRSA isolates of positive participants (N=15)

MRSA-positive participants (N=15)						
Strain number	**MLVA type**	**MLVA complex**	**PVL**	**Residing in the Netherlands since**	**Number of times MLVA type was diagnosed in the Amsterdam region within one year of date of isolation***	**Number of times MLVA type was diagnosed in the Netherlands within one year of date of isolation***
1	MT2502	NC	positive	1995	1	1
2	MT0121	MC0005	negative	2018	1	1
3	MT4112	MC0088	negative	2018	1	1
4	MT6237	MC0008-NC	negative	2016	1	1
5	MT0602	MC0005	negative	2016	8	27
6	MT0486	MC0022	negative	2012	1	11
7	MT0489	MC0254	negative	2014	16	71
8	MT2307	MC0005	negative	2018	1	1
9	MT6179	MC1933	negative	2016	1	2
10	MT0491	MC0022	negative	1990	16	114
11	MT2129	MC0282	positive	2018	1	2
12	MT0321	MC0008	negative	2016	6	35
13	MT0602	MC0005	negative	2009	2	2
14	MT0012	MC0005	negative	2016	1	1
15	MT0432	MC0435	positive	2016	1	1

* Date of isolation refers to the isolation of the MRSA strain from the participant included in this study. This number also includes the strain from the participant.
Abbreviations: MRSA – methicillin-resistant *Staphylococcus aureus*; PVL – Panton-Valentine leukocidin; MLVA – Multiple Loci Variable Number Tandem Repeat Analysis; MT – MLVA-type; NC – nearest complex; MC – MLVA-complex

Netherlands within one year before participant MRSA strains were isolated. MLVA cluster analysis of the 15 strains obtained in the present study and other strains isolated in the laboratory of the Public Health Service of Amsterdam showed no genetic relationship between strains from participants with one exception (strains 5 and 13, table 2). A few strains were included in larger clusters consisting of other, previously isolated strains.

DISCUSSION

In this cross-sectional study among patients attending an NGO health care facility for GP consultations in Amsterdam, the Netherlands, we found a prevalence of 2.0% for nasal MRSA carriage among undocumented migrants and uninsured legal residents. Prevalence did not differ between the two groups. Sociodemographic characteristics, migration history and other potential risk factors for MRSA were not associated with MRSA carriage. Three participants harbored PVL-positive isolates.

The prevalence of nasal MRSA carriage among undocumented migrants and uninsured legal residents from Amsterdam was higher than that reported for the general Dutch population(<1%) (5, 8, 9). This finding may partly reflect the prevalence of MRSA carriage in the participants' country of origin or in countries through which they travelled in transit to the Netherlands. Another possibility is that MRSA was transmitted between undocumented migrants and uninsured legal residents during their stay in the Netherlands. The fact that 14 different MLVA types were found in the MRSA-positive participants would argue for the former hypothesis. Nevertheless, some MLVA types were also frequently identified in other isolates from inhabitants of the Netherlands (i.e. MT0602, MT0489, MT0491 and MT0321). In addition, we did find other MLVA types (MT0121, MT6237, MT2307 and MT0012) that had not been isolated in other persons belonging to the well-known, worldwide occurring MLVA-complexes MC0005 and MC0008. Nevertheless, MLVA types could represent subtle differences from the MLVA-complexes frequently occurring in the Netherlands and might not be recognizably different. It is unknown from routine surveillance data whether these MLVA types are specifically found in migrants.

A meta-analysis on antimicrobial resistance among migrants in Europe found a pooled 8% prevalence of MRSA carriage (11). A previous retrospective study analyzing screening cultures from asylum seekers who recently arrived in the Netherlands similarly observed a 6% prevalence of nasal MRSA carriage (10). This prevalence would be almost threefold higher compared to that found in our study. Several hypotheses could explain the varying prevalence of MRSA carriage across studies. There could be differences between asylum seekers and undocumented migrants or uninsured legal residents

with respect to housing conditions, country of origin or socioeconomic status. Asylum seekers legally entering the Netherlands, as a result of applying for asylum through the centralized application system, are typically accommodated in an asylum center pending their application. Apart from other (indirect) transmission routes, MRSA is known to spread through skin-to-skin contact in places where crowding and contact occur, such as in schools, camps, gyms, prisons, and possibly asylum centers(29). Alternatively, MRSA could spread during crowded travel to Europe, such as on refugee boats or in tent camps. Nonetheless, a previous report observed 56 different MLVA types among 104 strains harbored among asylum seekers and considering the wide distribution of countries of origin in their study, the presence of MRSA would be more linked to migrants' geographical origin than transmission between asylum seekers(10).

It should be noted that the prevalence of nasal MRSA carriage among rejected asylum seekers in our study (2/135=1.5%, 95%CI 0.2% to 5.3%) was lower than the prevalence of nasal MRSA carriage found in a previous Dutch study of asylum seekers (5.6%) (10). Asylum seekers whose applications for asylum have been rejected are probably more likely to have been in the Netherlands longer than those currently seeking asylum. Although the median duration needed to clear MRSA carriage is not well known(30-32), MRSA acquired from their country of origin may have cleared spontaneously in our study population by the time they were screened. Another study has demonstrated that asylum seekers living in the Netherlands for more than one year had a lower prevalence of MRSA carriage than recently arrived migrants, thereby providing further evidence for this claim(33). Yet, at a 5.1% prevalence in these longer stay migrants, MRSA carriage would still be higher than found in our study or in the general Dutch population.

We did not find any statistically significant risk factors for nasal MRSA carriage, although we did find that individuals with current antibiotic use or ever injecting drug use tended to have a higher prevalence of MRSA carriage. Antibiotic use(34) and injecting drug use(35) are known risk factors for MRSA, in addition to, among others, recent admittance to or treatment in a foreign hospital and working with livestock(6). We were unable to confirm these latter findings, mainly owing to the lack of power in our study.

In the Netherlands, the MRSA search and destroy policy ensures that high-risk groups for MRSA are actively screened and pre-emptively isolated upon hospital admission(6). In 2015, the working group on infection prevention (WIP) additionally advised screening individuals who lived in an asylum center in the previous two months for MRSA carriage upon hospital admission(36). The relatively low MRSA prevalence of 2.0% found in our study compared to the prevalences found in acknowledged high-risk groups for carriage would suggest that screening undocumented migrants and uninsured legal residents

admitted to the hospital would be unjustified. However, notwithstanding the small sample size and limited power to identify significant determinants, studies are needed to confirm our findings.

The main strength of our study is that we included diverse populations that have not yet been considered in previous studies. We were able to reach many, generally hard-to-reach, undocumented migrants and uninsured legal residents of Amsterdam and as the study was conducted in six different languages, a broader geographical range of migrants' country of origin could be included.

However, some limitations need to be addressed. First, as patients were required to have understood one of the six study languages and were taking part in the study with additional HBV, HCV, and HIV screening, the study population was restricted to a convenience sample. Furthermore, Kruispost is a charity-based organization and in order to reduce study costs, we deliberately chose not to have a research associate present for inclusion at all times. The non-random, selective dates of inclusions could have contributed to a lower response. Both the convenience sample and low response might introduce selection bias, which could limit the generalizability of not only all patients at Kruispost, but also of the entire population of undocumented migrants and uninsured legal residents in Amsterdam. Opt-out options were available for MRSA, HBV, HCV and HIV screening; thus the screening for any specific infection was unlikely to influence the attractiveness of this study and reduce the response. Second, over forty percent of patients declined participation and only a small proportion of non-responders completed the short questionnaire on reasons for non-participation. Therefore, it is questionable whether the latter proportion is representative of all non-responders. Moreover, based on this small proportion of non-responders, non-response might be selective. It is unknown to what extent selective non-response and its representativeness for non-responders would have biased our results. Third, more recent migrants were less likely to participate in the study. Since MRSA carriage can spontaneously clear, the prevalence found in our study might be an underestimation compared to that from a study including more recent migrants with potentially more recent exposure to MRSA from their home country. Fourth, it was possible that the pattern of missing data was non-monotonic, potentially biasing our results. Most missing data were observed with respect to recent antibiotic use, but we do not know whether missingness was associated with recent antibiotic use. Fifth, we were unable to reach our target of 1000 participants, causing a lower absolute number of participants with MRSA carriage. Therefore, our study has limited power to evaluate determinants of MRSA carriage. Sixth, since we only assessed nasal MRSA carriage, it is possible that patients carrying MRSA in other locations were missed. Patients

with current antibiotic use may have also had false-negative results(37). These factors could have resulted in an underestimation of the true prevalence of MRSA.

Conclusions

To the best of our knowledge, this is the first study to examine nasal MRSA carriage among undocumented migrants and uninsured legal residents. Identifying groups with an increased risk of MRSA carriage could lessen the public health consequences of antimicrobial-resistant microorganisms in an interconnected world. Bearing the limited study sample in mind, we show that even though our study population has a higher MRSA prevalence than the general Dutch population, the prevalence is lower than that found in many other studies among migrants and asylum seekers. Future studies should confirm the relatively low prevalence of MRSA carriage among undocumented migrants and uninsured legal residents and may explore explanations for differences between this population and asylum seekers.

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Conflicts of interest

The authors declare that they have no competing interests related to the study.

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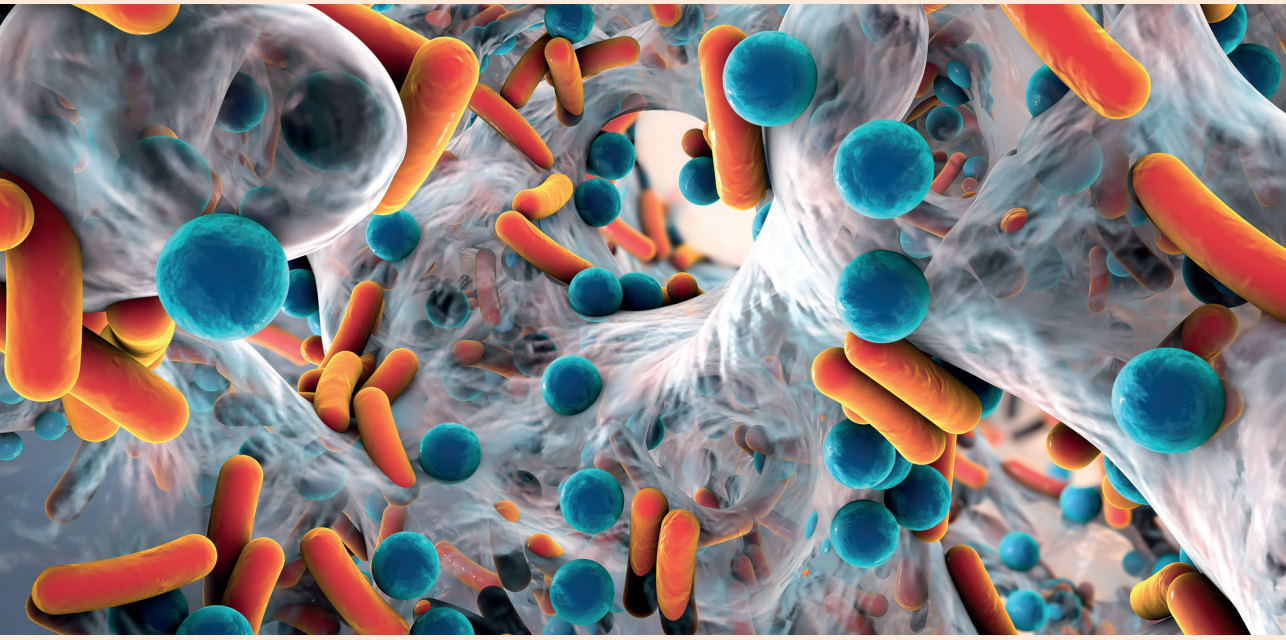
Supplementary table 1. Characteristics of included participants (N=784) versus patients who refused participation (but completed a short questionnaire on basic characteristics, N=195) in Amsterdam, the Netherlands, October 2018 - October 2019

	Included participants (N=784)		Non-included patients (N=195)		P-value*
	n	%	n	%	
Demographics					
Sex					.347
Male	457	58%	109	56%	
Female	326	42%	85	44%	
Other	1	0.1%	1	0.5%	
Age					.987
<35 years	262	33%	66	34%	
35-49 years	309	39%	79	41%	
50-64 years	187	24%	44	23%	
≥65 years	26	3%	6	3%	
Region of birth					<.001
Europe	40	5%	40	21%	
Asia	234	30%	54	28%	
Africa	276	35%	41	21%	
North/South America	233	30%	60	31%	
Year of leaving country of origin					.002
<2010	346	45%	67	36%	
2010-2017	335	43%	79	42%	
≥2018	93	12%	41	22%	
Year of arrival in the Netherlands					.131
<2010	287	37%	61	32%	
2010-2017	316	41%	72	38%	
≥2018	173	22%	55	29%	
Way of entering the Netherlands					<.001
Expired tourist/working/student visa	413	53%	98	52%	
Rejected asylum seeker	139	18%	23	12%	
EU citizen	47	6%	35	19%	
Other/unknown**	176	23%	32	17%	

* Differences in variables by MRSA carriage were assessed using a Fisher exact test for categorical data.
** Includes illegally crossing borders, legally, family visa, Schengen visa and work

Supplementary table 2. Reasons for non-participation among patients who completed a short questionnaire on basic characteristics (N=195) in Amsterdam, the Netherlands, October 2018 - October 2019

Reason	n	%
Time constraints	28	14%
Refused blood draw	40	21%
Afraid of test result	3	2%
Unwilling to participate in any form of research	4	2%
Afraid of being evicted from the Netherlands	2	1%
Not interested	26	13%
Experiencing language barriers in study information	19	10%
Recently tested for HBV/HCV/HIV	45	23%
Other reasons	22	11%



Part II

Prevalence of extended spectrum β-lactamase (ESBL) producing *Enterobacterales* among specific groups in Amsterdam, the Netherlands

5

High carriage of ESBL-producing *Enterobacterales* associated with sexual activity among men who have sex with men

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ABSTRACT

Background

Extended spectrum β-lactamase Enterobacteriaceae (ESBL-E) might be sexually transmitted. Men who have sex with men (MSM) have different sexual behavior than the general population and thus might be at risk for ESBL-E carriage. We determined the prevalence of ESBL-E carriage and its association with sexual behavior among MSM in Amsterdam, the Netherlands.

Methods

We screened 583 HIV-positive and HIV-negative MSM of the Amsterdam Cohort Study for rectal ESBL-E carriage between April-December 2018. Participants completed a self-administered questionnaire on (sexual) behavior and risk factors for antibiotic resistance. The proportion with ESBL-E carriage was compared by number of sex partners using logistic regression and across clusters of sexual behaviors with steady and casual partners, separately, using latent class analyses; all adjusted for recent antibiotic use, travel and hospitalization.

Results

16.3% (95%-confidence interval (95%CI)=13.4-19.5) tested ESBL-E positive. The odds of ESBL-E carriage increased as number of sexual partners increased (adjusted odds ratio per ln(partner+1), 1.57, 95%CI=1.26-1.94; p<0.001). There was no association between ESBL-E carriage and sexual behavior with steady partners. Compared to participants in the 'no sex with casual partner' cluster, adjusted odds of being ESBL-E positive were 2.95-fold higher (95%CI=1.52-5.80) for participants in the 'rimming and frottage' cluster (p=0.001) and 2.28-fold higher (95%CI=0.98-5.31) for participants in the 'toy use and fisting' cluster (p=0.056).

Conclusions

ESBL-E prevalence in MSM is higher than the overall Dutch population, likely due to sexual transmission with casual partners. This implies that sexually-active MSM should be considered a risk group for ESBL-E carriage.

BACKGROUND

Antimicrobial resistance (AMR) is a growing, global problem and an increasing threat to public health (1). An important resistance mechanism is production of extended spectrum β-lactamase (ESBL), which is an enzyme able to disintegrate commonly used β-lactam antibiotics, such as penicillins and extended-spectrum cephalosporins. (2). Worldwide, ESBL-producing Enterobacteriaceae (ESBL-E) have been increasingly found in hospital specimens and in samples of community-acquired infections (3-7). Initiating effective antibiotic treatment is often delayed in patients with ESBL-E infections, leading to increased mortality (8, 9).

ESBL-E resides mainly in the gastrointestinal tract and infection with ESBL-E is strongly associated with preceding carriage (3). Risk factors associated with ESBL-E colonization and infection include recent antibiotic use, travel to Asia and hospitalization (2, 10-16). ESBL-E is predominantly transmitted via fecal-oral contact (17). Recent studies have also suggested possible sexual transmission of ESBL-E. Reinheimer *et al.* observed an increased prevalence of ESBL-E among HIV-positive compared to HIV-negative men, which was purportedly due to sexual transmission of ESBL-E in HIV-positive men (18). Other studies reported clusters of ESBL-producing *Shigella* among men who have sex with men (MSM), which, again, was thought to be associated with sexual transmission (19-21). Given these findings, MSM might be at increased risk for ESBL-E colonization and subsequent infections.

To further substantiate the role of sexual transmission of ESBL-E, we measured the prevalence of rectal ESBL-E carriage in MSM participating in the Amsterdam Cohort Studies (ACS), Amsterdam, the Netherlands, and determined its association with sexual behavior.

METHODS

Study design and population

The ACS is an open, on-going, prospective cohort study among HIV-positive and HIV-negative MSM, which was initiated in 1984 to investigate the prevalence, incidence, and risk factors of HIV and other sexually transmitted infections (22). The ACS recruits men who had sex with at least one other man in the preceding 6 months and live in the Amsterdam area or are involved in MSM-related activities in Amsterdam. Participants are recruited via 'chain referral sampling' (participants recruiting other participants) and 'convenience sampling' (recruitment via online advertisements on gay dating

apps or via outreach activities at MSM meeting places). Participants visit the Public Health Service of Amsterdam every 3-6 months. At each visit, participants complete a self-administered questionnaire on sexual behavior in the preceding 6 months and are tested for HIV-1 (HIV-negative MSM only), syphilis and pharyngeal, urogenital and anal chlamydia and gonorrhea.

During study visits scheduled between April-December 2018, participants were asked to provide a self-collected anal swab (E-Swab®, Copan Diagnostics Inc.) to test for ESBL-E carriage and were additionally asked to respond to questions on antibiotic use and (potential) risk factors for carriage of AMR bacteria (*Supplementary Materials*). Questions were based on the HELIUS questionnaire on risk factors for infectious diseases (23).

Testing for ESBL-E carriage

Extended spectrum ß-lactamase (ESBL) producing strains are resistant for broad spectrum cephalosporins, such as ceftazidime and cefotaxime. ESBL forming strains were detected by the Public Health laboratory (PHL) in Amsterdam by culturing anal swabs (Fecalswab Copan Diagnostics Inc.) For ESBL screening, anal swabs were cultured overnight in enrichment brain heart infusion broth supplemented with 16 mg/l amoxicilline (Mediaproducts BV) at 37°C. For prescreening, the broth was subcultured on McConkey agar plates (bioMérieux, Marcy l'Étoile, France) for 24 hours. One 10 µg ceftazidime disc and one 5 µg cefotaxime disc (Rosco, Taastrup, Denmark) were placed on the McConkey agar about 2.5 cm apart before incubation. Colonies growing on the McConkey agar plate within a zone of <20 mm for ceftazidime and/or <22 mm for cefotaxime (EUCAST detection of resistance mechanisms v1.0, www.eucast.org) were tested for susceptibility (MIC) using Vitek-2XL system (bioMérieux, Marcy l'Etoile, France). Determination was performed by MALDI Biotyper (Bruker). The Vitek-2XL system indicates probable presence of ESBL. To confirm the presence of ESBL, the ESBL gradient test method according to EUCAST guidelines was performed by testing 3 combinations of e-tests (bioMérieux, Marcy l'Etoile, France) ceftazidime, cefotaxime, and cefepime with and without clavulanic acid on a Mueller Hinton ll agar (Becton Dickinson, US). The level of resistance is calculated by the ratio of MICs, without clavulanic acid versus the MICs with clavulanic acid. For ESBL confirmation, a ratio of ≥8 for at least 1 of the 3 e-tests is indicated (EUCAST detection of resistance mechanisms v1.0, www.eucast.org).

Study variables

The primary outcome was rectal carriage of ESBL-E. Age, country of birth and educational level were included as socio-demographic variables. Known risk factors for infection/carriage with AMR bacteria included antibiotic use, travelling to ESBL-E endemic countries and hospital admittance; all within the preceding 6 months (2, 10-16). Potential risk

factors for infection/carriage with AMR bacteria included HIV status, sexual behavior, having ever not completed antibiotic treatment, having ever saved antibiotics for later use, pet ownership (cat, dog, and/or horse), meat consumption, and being employed in patient care or aviation (3, 16, 18, 24, 25). Travel history was categorized according to travel to specific continents and travel to low (prevalence<10%), medium (prevalence 10-25%), and high (prevalence>25%) ESBL-E endemic countries (13, 26). The highest prevalence category was assigned to individuals traveling to multiple countries with varying ESBL-E endemicity. Variables on sexual behavior in the preceding six months included: *with steady and casual partners* – having sex, number of sexual partners, insertive and receptive fellatio, insertive and receptive anal sex, active and passive rimming; *with casual partners* – active and passive frottage, active and passive fisting, active and passive sex with toys, and sharing toys. Casual partners included both known and anonymous sex partners.

Statistical analysis

All included variables were compared between ESBL-E positive and negative MSM using Pearson's χ^2 test or Fisher's exact test for categorical data and Mann-Whitney U test for continuous data. Unadjusted odds ratios (OR) comparing odds of ESBL-E positivity across levels of determinants, along with their 95% confidence intervals (95%-CI), were estimated using univariable logistic regression.

We constructed a series of multivariable models to assess the association between sexual behavior and ESBL-E carriage. To determine variables for adjustment, the relationship between sexual behavior and ESBL-E carriage would likely be confounded by antibiotic use in the past six months, travel to medium/high ESBL-E endemic country in the past six months and being admitted to the hospital in past six months (2, 10-16). Hence, we included these variables *a priori* in multivariable adjustment. We then evaluated the role of other potential confounders by additionally including socio-demographic characteristics and other potential risk factors with a *p*-value <0.2 in univariable analysis and removing those that became non-significant(*p*-value >0.05) in backward-stepwise fashion.

Due to the strong correlation between number of sex partners and partner-dependent sexual behaviors, we added the following components to three separate multivariable models: (i) number of sex partners, (ii) sexual behavior with steady partners and (iii) sexual behavior with casual partners. The OR for the association between number of sex partners and ESBL-E carriage was obtained by logistic regression, in which the number of sex partners, n, was modeled as $\ln(n+1)$. Since many of the sexual behaviors overlapped, we modeled sexual behavior with steady and casual partners, separately, as expressed

in latent classes (27). Variables used for identifying clusters of sexual behavior with steady partners included the following: insertive and receptive fellatio, anal sex and rimming. Variables used for identifying clusters of sexual behavior with casual partners included the following: insertive and receptive fellatio, anal sex, frottage, rimming, toy use and fisting. All variables considered were binomially distributed. We allowed the latent variable *c* to have *K* classes ($k = 1, 2, \ldots, K$). Using conditional maximum likelihood methods, we jointly modeled ESBL-status, covariates, and the conditional probability of performing specific sexual behavior given latent class *k*, along with class probabilities, by an intercept-only logistic regression model (for each sexual behavior with respect to latent class, a_{sk}) and multinomial model with *k*-specific intercepts (for ESBL-status and each covariate with respect to latent class, a_{sk}). The OR and 95%CI for the association between latent class and ESBL-status were obtained from the parameter estimate corresponding to ESBL-status. For both models on sexual behavior with steady and causal partners, the number of classes *k* were sequentially increased. The Bayesian information criterion (BIC) was calculated for each of these models to assess model fit and the final model was based on the one with the lowest BIC. We estimated latent class models using the 'gsem' command in STATA.

To estimate the distribution of individuals in each latent class, we used the conditional likelihood from which the *a posteriori* probability of an individual *i* belonging to each class *k*, π_{ik}, can be calculated. MSM were assigned to the class in which they had the highest probability of membership. The degree to which class membership could be separated was examined using a variable-specific entropy contribution statistic, ranging from 0 to 1, with higher levels individuals more distinct separation of classes (28). Of note, assigned classes were not used to estimate the OR and 95%CI of the association between sexual behavior clusters and ESBL-E membership.

All analyses were conducted with STATA v15.1 (StataCorp., College Station, Texas, USA). A p-value <0.05 was defined as statistically significant.

Ethical approval and informed consent

The Medical Ethical Committee of the Amsterdam UMC approved the ACS (NL49748.018.14). Participation is voluntary and each participant gave written informed consent at study enrollment.

RESULTS

In total, 695 MSM had at least one ACS study visit between April-December 2018. Of them, 583 (84%) were tested for ESBL-E carriage and completed the questionnaire and were hence included in analysis. Median age was 43 (interquartile range (IQR) 34-50) years (*Table 1*). The majority was born in the Netherlands (n=482, 83%) and had a college degree or higher (n=441, 76%). 540 (93%) were HIV-negative and 43 (7%) were HIV-positive. Almost a quarter (n=141, 24%) reported use of antibiotics in the preceding 6 months. Only 25 (4%) participants reported never have used antibiotics. Among participants who ever used antibiotics, 56 (12%) reported not having finished their antibiotics at least once. Recent travel to a medium or high endemic country was reported by 219 (38%) and 172 (29%) participants, respectively, and recent hospitalization by 69 (12%) participants.

Prevalence of ESBL-producing *Enterobacteriaceae* carriage and variables associated with carriage

ESBL-E carriage was detected in 95 participants (16.3%; 95%-CI 13.4-19.5%). Among these participants, 139 unique ESBL producing isolates were cultured, of which 130 (94%) were identified as *Escherichia coli*, 6 (4%) as *Klebsiella pneumoniae*, 2 (1%) as *Enterobacter cloacae* and 1 (1%) as *Escherichia hermannii*. There was no difference in age, country of birth, educational level or HIV-status between ESBL-E positive and negative participants (*Table 1*). ESBL-E positive participants more often used antibiotics in the preceding 6 months compared to ESBL-E negative participants (33% vs. 23%, p=0.036). Other known or potential risk factors for AMR, such as travel history, hospitalization, meat consumption, being a pet owner or being employed in patient care, did not differ between groups. Being employed as cabin personnel or a pilot was associated with being ESBL-E positive (n=9 (9%) versus n=19 (4%); p=0.020).

The median number of sexual partners in the preceding 6 months was 12 (IQR 4-28) in ESBL-E positive participants, which was significantly higher than in ESBL-E negative participants (median 5 (IQR 1-13); p<0.001). Compared to ESBL-E negative participants, ESBL-E positive participants more frequently reported insertive fellatio (n=64 (75%) vs. n=292 (63%); p=0.032), receptive fellatio (n=68 (80%) vs. n=291 (63%); p=0.002), insertive anal sex (n=58 (69%) vs. n=243 (52%); p=0.005), receptive anal sex (n=52 (62%) vs. n=214 (46%), p=0.008) and receptive rimming (n=56 (66%) vs. n=224 (49%); p=0.003) with casual partners. A comparable proportion of ESBL-E positive and negative participants had a steady partner, and there were no differences in sexual behaviors with steady partners between both groups.

Table 1. Sociodemographics, risk factors for antibiotic resistance and sexual behavior among 583 MSM participating in the Amsterdam Cohort Studies between April and December 2018, Amsterdam, the Netherlands, stratified by ESBL-E carriage.

	Total (N=583)		ESBL-E negative MSM (N=488)		ESBL-E positive MSM (N=95)		p-value*
	n	%	n	%	n	%	
Demographics							
Age in years, median [IQR]	43	[34-50]	43	[34-50]	42	[30-51]	.355
Country of birth							.326
The Netherlands	482	83%	407	84%	75	80%	
Outside the Netherlands	97	17%	78	16%	19	20%	
Education							.071
No college degree	140	24%	124	26%	16	17%	
College degree or higher	441	76%	362	74%	79	83%	
Known risk factors for ABR							
Antibiotic use in past six months	141	24%	110	23%	31	33%	.036
Travel history in past six months†							.442
No travel	114	20%	94	19%	20	21%	
Travel to low-endemic countries	78	13%	65	13%	13	14%	
Travel to medium-endemic countries	219	38%	190	39%	29	31%	
Travel to high-endemic countries	172	29%	139	28%	33	35%	
Travel history in past six months§							
Western Europe	297	51%	247	51%	50	53%	.719
Northern Europe	92	16%	77	16%	15	16%	.998

Table 1. Sociodemographics, risk factors for antibiotic resistance and sexual behavior among 583 MSM participating in the Amsterdam Cohort Studies between April and December 2018, Amsterdam, the Netherlands, stratified by ESBL-E carriage. *(continued)*

	Total (N=583)		ESBL-E negative MSM (N=488)		ESBL-E positive MSM (N=95)		p-value*
	n	%	n	%	n	%	
Eastern Europe	43	7%	33	7%	10	11%	.199
Southern Europe	232	40%	196	40%	36	38%	.679
Asia	93	16%	74	15%	19	20%	.239
Northern America	49	8%	41	8%	8	8%	.995
Southern America	30	5%	24	5%	6	6%	.573
Central America	18	3%	14	3%	4	4%	.514
Caribbean	14	2%	12	2%	2	2%	.999
Sub-Saharan Africa	26	4%	20	4%	6	6%	.338
Northern Africa	9	2%	6	1%	3	3%	.168
Oceania	6	1%	4	1%	2	2%	.254
Admitted to Dutch hospital in past six months	62	11%	51	10%	11	12%	.744
Admitted to foreign hospital in past six months	8	1%	8	2%	0	0.0%	.365
Potential risk factors for ABR							
HIV status							.666
Negative	540	93%	451	92%	89	94%	
Positive	43	7%	37	8%	6	6%	
Did not finish antibiotic treatment							.921
Yes, regularly	1	0.2%	1	0.2%	0	0.0%	

Table 1. Sociodemographics, risk factors for antibiotic resistance and sexual behavior among 583 MSM participating in the Amsterdam Cohort Studies between April and December 2018, Amsterdam, the Netherlands, stratified by ESBL-E carriage. *(continued)*

	Total (N=583)		ESBL-E negative MSM (N=488)		ESBL-E positive MSM (N=95)		p-value*
	n	%	n	%	n	%	
Yes, occasionally	55	11%	46	11%	9	11%	
No, never	430	84%	357	84%	73	86%	
Not applicable (no antibiotic use)	25	5%	22	5%	3	4%	
Saved antibiotics for later¢							.103
Yes, occasionally	20	4%	14	3%	6	7%	
No, never	45	9%	41	9%	4	5%	
Meat consumption							.242
Regularly	449	77%	381	78%	68	72%	
Sometimes	103	18%	84	17%	19	20%	
Never	31	5%	23	5%	8	8%	
Cat owner	110	19%	89	18%	21	22%	.378
Dog owner	62	11%	49	10%	13	14%	.292
Horse owner	8	1%	5	1%	3	3%	.127
Employed in patient care	63	11%	54	11%	9	9%	.647
Employed as cabin personnel or pilot	28	5%	19	4%	9	9%	.020
Sexual behavior (past 6 months)							
Number of sex partners, median [IQR]	6	[1-15]	5	[1-13]	12	[4-28]	<.001

Table 1. Sociodemographics, risk factors for antibiotic resistance and sexual behavior among 583 MSM participating in the Amsterdam Cohort Studies between April and December 2018, Amsterdam, the Netherlands, stratified by ESBL-E carriage. *(continued)*

	Total (N=583)		ESBL-E negative MSM (N=488)		ESBL-E positive MSM (N=95)		p-value*
	n	%	n	%	n	%	
Steady partner(s)							
Having a steady partner	339	62%	289	62%	50	60%	.615
Insertive fellatio (receiving blowjob)	271	49%	229	49%	42	50%	.913
Receptive fellatio (giving blowjob)	273	50%	231	50%	42	50%	.971
Insertive anal sex	171	31%	142	31%	29	35%	.483
Receptive anal sex	175	32%	145	31%	30	36%	.427
Rimming partner	163	30%	138	30%	25	30%	.997
Getting rimmed	151	28%	125	27%	26	31%	.449
Casual sex partner(s)							
Having a casual partner	414	76%	343	74%	71	85%	.040
Insertive fellatio (receiving blowjob)	356	65%	292	63%	64	75%	.032
Receptive fellatio (giving blowjob)	359	66%	291	63%	68	80%	.002
Insertive anal sex	301	55%	243	52%	58	69%	.005
Receptive anal sex	266	49%	214	46%	52	62%	.008
Rimming partner	259	47%	211	46%	48	56%	.067
Getting rimmed	280	51%	224	49%	56	66%	.003
Fisting partner	60	11%	50	11%	10	12%	.781
Getting fisted	38	7%	29	6%	9	11%	.143

Table 1. Sociodemographics, risk factors for antibiotic resistance and sexual behavior among 583 MSM participating in the Amsterdam Cohort Studies between April and December 2018, Amsterdam, the Netherlands, stratified by ESBL-E carriage. *(continued)*

	Total (N=583)		ESBL-E negative MSM (N=488)		ESBL-E positive MSM (N=95)		p-value*
	n	%	n	%	n	%	
Inserting toys in partner	70	13%	56	12%	14	17%	.255
Getting toys inserted	70	13%	57	12%	13	16%	.409
Sharing toys	24	5%	20	5%	4	5%	.776

* ESBL-E carriage groups were compared using Pearson's χ^2 test or when expected values were <5 in one or mole cells, Fisher's exact test for categorical data and Kruskall-Wallis test for continuous data.

† Low, medium and high endemic countries were based on studies by Arcilla (2016) and Doi (2017).

§ Compared to not traveling to a given region.

ϕ Among those who ever did not finish their antibiotic treatment.

Abbreviations: ABR = antibiotic resistance; ESBL-E = extended spectrum β-lactamase-producing Enterobacteriaceae; MSM = men who have sex with men; HIV = human immunodeficiency virus.

Missings: country of birth 4; education 2; not finishing antibiotic treatment 72; saving antibiotics for later 63; number of sex partners 45; having steady partner 35; insertive fallatio with steady partner 35; receptive fallatio with steady partner 35; insertive anal sex with steady partner 36; receptive anal sex with steady partner 36; rimming steady partner 35; getting rimmed by steady partner 35; having casual partner 36; insertive fallatio with casual partner 36; receptive fallatio with casual partner 36; insertive anal sex with casual partner 36; receptive anal sex with casual partner 36; rimming casual partner 36; getting rimmed by casual partner 37; fisting casual partner 39; getting fisted by causal partner 38; inserting toys in casual partner 38; getting toys inserted by casual partner 39; sharing toys with casual partner 90.

The unadjusted odds ratios comparing odds of ESBL-E positivity across levels of determinants can be found in *Supplementary Table 1*.

Association between extended beta-lactamase producing *Enterobacteriaceae* carriage and clusters of sexual behavior

All multivariable models evaluating the association between ESBL-E carriage and sexual behavior were adjusted by antibiotic use, travel history and hospitalization in the preceding 6 months (all pre-defined; no other variables were retained in backwards selection). In the first model, the odds of ESBL-E carriage increased as number of sexual partners increased [adjusted OR per ln(partner+1), 1.57, 95%-CI 1.26-1.94; p<0.001; *Table 2*]

Latent class analysis (LCA) identified three different clusters for sexual behavior with steady partners (*Supplementary Table 2*), which we labeled as follows: (1) 'no sex with steady partner' (including participants without a steady partner), (2) 'only fellatio', and (3) 'fellatio, rimming and anal sex'. In total, 265 (45%) participants were assigned to the 'no sex with steady partner' cluster, 129 (22%) to the 'only fellatio' cluster, and 154 (26%) to the 'fellatio, rimming and anal sex' cluster. For sexual behavior with steady partners, the variable-specific entropy indicating degree of class separation was 0.89 and the distributions of a posteriori probabilities according to participants' assigned class membership are shown in *Supplementary Figure 1*. From the latent class model, none of the identified clusters were associated with ESBL-E carriage in multivariable analysis (*Table 2*).

For sexual behavior with casual partners, LCA revealed four different clusters (*Supplementary Table 2*), which were labeled as: (1) 'no sex with casual partner' (including participants without a casual partner), (2) 'fellatio and anal sex', (3) 'fellatio, anal sex, rimming and frottage', and (4) 'multiple behaviors including toy use and fisting'. 181 (31%) participants were assigned to the 'no sex with casual partner' cluster, 134 (23%) to the 'fellatio and anal sex' cluster, 165 (28%) to the 'fellatio, anal sex, rimming and frottage' cluster, and 67 (11%) to the 'multiple behaviors including toy use and fisting' cluster. For sexual behavior with casual partners, the variable-specific entropy indicating degree of class separation was 0.74 and the distributions of a posteriori probabilities according to participants' assigned class membership are shown in *Supplementary Figure 2*. From the latent class model, compared to participants in the 'no sex with casual partner' cluster, the adjusted odds of being ESBL-E positive was 2.95-fold higher (95%-CI 1.52-5.80) for participants in the 'fellatio, anal sex, rimming and frottage' cluster (p=0.001) and 2.28-fold higher (95%-CI 0.98-5.31) for participants in the 'multiple behaviors including toy use and fisting' cluster (p=0.056) (*Table 2*).

Table 2. Univariable and multivariable logistic regression analyses of sexual behavior variables and their association with ESBL-E carriage among 583 MSM participating in the Amsterdam Cohort Studies between April and December 2018, Amsterdam, the Netherlands.

	Univariable associations			Multivariable model with number of sexual partners[†]			Multivariable model with sexual behavior cluster with steady partners[†]			Multivariable model with sexual behavior cluster with casual partners[†]		
	OR	95% CI	p-value	aOR	95% CI	p-value	aOR	95% CI	p-value	aOR	95% CI	p-value
Number of sexual partners[¢]	1.56	1.26-1.93	<.001	1.57	1.26-1.94	<.001						
Sexual behavior cluster with steady partners*												
(1) no sex with steady partner	Ref						Ref					
(2) only fellatio	1.09	0.61-1.95	.760				1.10	0.61-1.98	.745			
(3) fellatio, rimming and anal sex	0.99	0.57-1.72	.965				1.02	0.57-1.84	.946			
Sexual behavior cluster with *casual* partners*												
(1) no sex with casual partner	Ref									Ref		
(2) fellatio and anal sex	1.70	0.84-3.45	.139							1.88	0.87-3.67	.116
(3) fellatio, anal sex, rimming and frottage	2.98	1.59-5.60	.001							2.97	1.52-5.80	.001
(4) multiple behaviors including toy use and fisting	2.48	1.12-5.49	.025							2.28	0.98-5.31	.056

[¢] Included as ln(number of sex partners + 1).
*Clusters are defined as latent classes of like sexual behavior, established separately for steady partners and casual partners.
[†] Adjusted for antibiotic use, travel history, hospitalization in preceding 6 months.
Abbreviations: OR = odds ratio; CI = confidence interval; aOR = adjusted odds ratio.
Missings: number of sexual partners 45; sexual behavior with steady partners 35; sexual behavior with casual partner 36.

DISCUSSION

The prevalence of ESBL-E in our relatively large cohort of MSM was 16.3%. After adjustment for recent antibiotic use, traveling and hospitalization, ESBL-E carriage was moreover associated with a high number of sex partners and practicing certain sexual behaviors with casual partners, especially those involving rimming. Of note, ESBL-E carriage was also higher in participants who recently used antibiotics.

ESBL-E prevalence in the general Dutch population has been reported to be 5.0% (95%-CI 3.4-6.6) in 2014-2016, after adjustment for age, sex, ethnicity and degree of urbanization (25). In 2011, Reuland et al. estimated the ESBL-E prevalence at 8.6% (95%-CI 7.3-10.0) in the general population of Amsterdam, the Netherlands (16). Several other studies have also provided ESBL-E prevalence among specific subgroups, such as farmers, travelers, patients visiting the general practitioner, households with young children or people living in either rural or urban areas (13, 16, 29-31). The prevalence in these studies ranged from 4% in households with young children to 51% among people with very recent travel to Asia (13, 31). These findings together would suggest that the 16.3% prevalence of rectal ESBL-E carriage in this cross-sectional study among MSM is remarkably higher than in the overall Dutch or Amsterdam population, but lower compared to other groups at increased risk, such as travelers to high ESBL-E endemic regions. While acknowledging that ESBL-E carriage has been shown to be increasing over recent years (3-7), our estimates might not be directly comparable to data from several of the mentioned studies conducted during earlier years.

Our study found an association between ESBL-E carriage and increasing number of sex partners in the preceding 6 months. By using LCA models, we attempted to identify sexual behaviors that might shed light on this association. This analysis revealed that the more sexual techniques participants engaged in with casual partners, the stronger the association with ESBL-E carriage. An increased risk was especially seen in participants who reported rimming. There was a strong, but borderline insignificant association between the ‘multiple behaviors including toy use and fisting’ cluster with casual partners and ESBL-E carriage, whose lack in significance might be explained by the low level of power to detect an association (only 67 participants belonged to this cluster). One could argue that higher number of sex partners and/or practicing certain sex behaviors would lead to an increased prevalence of sexually transmitted infections (STIs) (22), which would require antibiotic treatment and thus higher proportions of antibiotic use. The selective pressure induced by antibiotics used to treat STIs, such as penicillin for the treatment of syphilis and ceftriaxone for the treatment of gonorrhea, would then bring rise to carriage of ESBL-E strains. We observed, however, that the increased risk

of ESBL-E carriage with higher numbers of sexual partners and practicing certain sex behaviors was independent of antibiotic use.

Due to the strong overlap in sexual behaviors and numbers of sexual partners (*Supplementary Table 3*), it is difficult to disentangle whether number of partners, practicing certain sex behaviors, or both are driving ESBL-E transmission. Although we were able to distinguish clusters of sexual behavior, their highly-correlated nature makes it difficult to pinpoint the specific route of sexual transmission of ESBL-E, if such an association exists. Nevertheless, this study is the first to explore the association between ESBL-E carriage and reported sexual behavior. Reinhiemer *et al.* did find an increased ESBL-E prevalence among HIV-positive men compared to HIV-negative individuals, which was explained by sexual transmission without any actual measure of sexual behavior in either group (18). Other research postulating sexual transmission of ESBL-E among MSM has been principally based on outbreak reports in MSM, in which measured sexual behavior was also missing (19-21). Our results provide unequivocally more concrete evidence to substantiate these previous claims that ESBL-E can be transmitted via sexual contact, rather than HIV-infection *per se*.

By using data from a large sample of MSM of the ACS, we were able to determine the prevalence of ESBL-E carriage and investigate its association with sexual behavior. Despite this strength, the current study has certain limitations. First, the ACS is based on a convenience sample, including predominantly highly-educated MSM who were born in the Netherlands and were sexually-active at time of enrolment. This group of MSM might therefore not represent the larger Amsterdam or Dutch MSM population. Second, not all potential risk factors for antibiotic resistance were measured in our study. Some studies reported, for example, that use of antacids and poor kitchen hygiene were associated with ESBL-E carriage (16, 25, 31). Since we intended to retain a higher proportion of respondents by providing a questionnaire with an acceptable length, we choose to keep the additional questionnaire as concise as possible. We might have therefore missed some risk factors in our study. Third, we used a backwards stepwise approach to arrive at our multivariable model, whereby certain biases due to model selection could arise. Nevertheless, the final model only included variables selected a priori that were likely to have confounded the relationship between sexual behavior and ESBL-E carriage, hence the influence of these biases were likely limited.

In conclusion, the ESBL-E prevalence among MSM is higher than in the overall Dutch population, which is likely explained by sexual transmission. Our data implies that sexually active MSM should be considered an at-risk group for ESBL-E carriage and might

warrant different isolation precautions and empirical antibiotic treatment in case of severe sepsis.

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Conflicts of interest

The authors declare that they have no conflicts of interest relevant to the manuscript submitted to the International Journal of Antimicrobial Agents.

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SUPPLEMENTARY MATERIAL

Questionnaire on recent antibiotic use and possible risk factors for carriage of ESBL.

Original questions in Dutch

Antibioticagebruik

1. Wanneer heeft u voor het laatst antibiotica gebruikt/toegediend gekregen?
   - ☐ Ik gebruik momenteel antibiotica
   - ☐ Minder dan 6 maanden geleden
   - ☐ Meer dan 6 maanden geleden
   - ☐ Nooit
   - ☐ Weet ik niet meer

2. De volgende vragen gaan over antibioticagebruik in de **afgelopen 6 maanden**.

   a) Hoeveel antibioticakuren heeft u gehad in de afgelopen 6 maanden? (NB: een antibioticakuur kan ook uit een eendaagse behandeling bestaan).
   - ☐ 1
   - ☐ 2
   - ☐ 3
   - ☐ 4 of meer

   b) Specificeer hieronder voor iedere antibioticakuur in de **afgelopen 6 maanden** (tot maximaal 4 kuren) waarvoor u deze heeft gebruikt.

   Antibioticakuur 1: _____
   Antibioticakuur 2: _____
   Antibioticakuur 3: _____
   Antibioticakuur 4: _____

   c) Specificeer hieronder voor iedere antibioticakuur in de **afgelopen 6 maanden** of u deze heeft voorgeschreven gekregen via een professioneel zorgverlener (zoals een arts).

Kuur 1:	**Kuur 2:**	**Kuur 3:**	**Kuur 4:**
☐ Ja	☐ Ja	☐ Ja	☐ Ja
☐ Nee	☐ Nee	☐ Nee	☐ Nee
☐ Weet ik niet meer	☐ Weet ik niet meer	☐ Weet ik niet meer	☐ Weet ik niet meer

d) Specificeer hieronder voor iedere antibioticakuur in de **afgelopen 6 maanden** of u instructies van een arts, verpleegkundige of apotheekmedewerker heeft ontvangen over de inname van uw antibioticakuur.

Kuur 1:	**Kuur 2:**	**Kuur 3:**	**Kuur 4:**
☐ Ja	☐ Ja	☐ Ja	☐ Ja
☐ Nee	☐ Nee	☐ Nee	☐ Nee
☐ Weet ik niet meer	☐ Weet ik niet meer	☐ Weet ik niet meer	☐ Weet ik niet meer

e) Specificeer hieronder waar u uw antibioticakuur in de **afgelopen 6 maanden** heeft verkregen.

Kuur 1:

☐ Apotheek
☐ Ziekenhuis
☐ SOA polikliniek
☐ Internet
☐ Via vriend of familielid
☐ Ik heb antibiotica gebruikt die ik thuis nog had liggen van een vorige kuur
☐ Weet ik niet meer
☐ Elders, namelijk _____

Kuur 2:

☐ Apotheek
☐ Ziekenhuis
☐ SOA polikliniek
☐ Internet
☐ Via vriend of familielid
☐ Ik heb antibiotica gebruikt die ik thuis nog had liggen van een vorige kuur
☐ Weet ik niet meer
☐ Elders, namelijk _____

Kuur 3:

☐ Apotheek
☐ Ziekenhuis
☐ SOA polikliniek
☐ Internet
☐ Via vriend of familielid
☐ Ik heb antibiotica gebruikt die ik thuis nog had liggen van een vorige kuur
☐ Weet ik niet meer
☐ Elders, namelijk _____

Kuur 4:

☐ Apotheek

☐ Ziekenhuis

☐ SOA polikliniek

☐ Internet

☐ Via vriend of familielid

☐ Ik heb antibiotica gebruikt die ik thuis nog had liggen van een vorige kuur

☐ Weet ik niet meer

☐ Elders, namelijk _____

f) Specificeer hieronder voor iedere antibioticakuur in de **afgelopen 6 maanden** of u deze heeft afgemaakt?

Kuur 1:

☐ Ja

☐ Nee, want _____

Kuur 2:

☐ Ja

☐ Nee, want _____

Kuur 3:

☐ Ja

☐ Nee, want _____

Kuur 4:

☐ Ja

☐ Nee, want _____

3. De volgende vragen gaan over antibioticagebruik gedurende uw hele leven.

a) Heeft u wel eens een antibioticakuur niet opgemaakt?

☐ Ja, regelmatig

☐ Ja, soms

☐ Nee, nooit → ga naar vraag 10

b) Hebt u wel eens een antibioticakuur die u niet had opgemaakt bewaard voor een volgende keer?

☐ Ja, regelmatig

☐ Ja, soms

☐ Nee, nooit

Risicofactoren

4. Bent u in de afgelopen 6 maanden in het buitenland geweest voor langer dan 24 uur?
   - ☐ Ja, namelijk in volgend(e) land(en)
     1) _____
     2) _____
     3) _____
     4) _____
     5) _____
     6) _____
     7) _____
     8) _____
     9) _____
     10) _____
   - ☐ Nee

5. Bent u in de afgelopen 6 maanden in een ziekenhuis opgenomen/behandeld geweest?
   - ☐ Ja, in Nederland
   - ☐ Ja, in het buitenland, namelijk in _____
   - ☐ Nee

6. Heeft u in de afgelopen 6 maanden vlees gegeten?
   - ☐ Ja, regelmatig
   - ☐ Ja, soms
   - ☐ Nee, nooit

7. Geef aan of u in de **afgelopen 6 maanden** in het bezit bent geweest van een van onderstaande dieren?
   - ☐ Kat(ten)
   - ☐ Hond(en)
   - ☐ Paard(en)
   - ☐ Geen van bovenstaande

8. Heeft u in de afgelopen 6 maanden in de zorg gewerkt waarbij u patiëntcontact had?
   - ☐ Ja, namelijk in een
     - ☐ Ziekenhuis
     - ☐ Verpleeg- of verzorgingshuis / woon-zorgcomplex
     - ☐ Hospice
     - ☐ Thuiszorginstelling
     - ☐ Huisartsenpraktijk

- ☐ Ambulancedienst
- ☐ GGD
- ☐ Anders, namelijk _____

☐ Nee

9. Heeft u in de afgelopen 6 maanden voor een luchtvaartmaatschappij gewerkt als cabinepersoneel of piloot?

   ☐ Ja

   ☐ Nee

Translated questions in English

Antibiotic use

1. When did you use antibiotics for the last time?

   ☐ I currently use antibiotics

   ☐ Less than 6 months ago

   ☐ More than 6 months ago

   ☐ Never

   ☐ I do not know

2. The following questions are about your antibiotic use in the **preceding 6 months**.

   a) How many courses of antibiotics did you have in the preceding 6 months? (PS: an antibiotic course can also be a one-day treatment).

   ☐ 1

   ☐ 2

   ☐ 3

   ☐ 4 or more

   b) Please specify for each course of antibiotics you used in the **preceding 6 months** (up to maximum 4 courses) the infection for which you used them.

   Course 1: _____

   Course 2: _____

   Course 3: _____

   Course 4: _____

   c) Please specify for each course of antibiotics you used in the **preceding 6 months** if this course was prescribed by a health care provider (like a doctor).

Course 1:	**Course 2:**	**Course 3:**	**Course 4:**
☐ Yes	☐ Yes	☐ Yes	☐ Yes
☐ No	☐ No	☐ No	☐ No
☐ I don't know	☐ I don't know	☐ I don't know	☐ I don't know

d) Please specify for each course of antibiotics you used in the **preceding 6 months** if you received instructions from a doctor, nurse or pharmacy assistant about how to use the antibiotics.

Course 1:	**Course 2:**	**Course 3:**	**Course 4:**
☐ Yes	☐ Yes	☐ Yes	☐ Yes
☐ No	☐ No	☐ No	☐ No
☐ I don't know	☐ I don't know	☐ I don't know	☐ I don't know

e) Please specify for each course of antibiotics you used in the **preceding 6 months** where you received these antibiotics.

Course 1:

☐ Pharmacy
☐ Hospital
☐ STI clinic
☐ Internet
☐ Via a friend or family member
☐ I have used antibiotics which I already had from a previous course
☐ I don't know
☐ Other, namely _____

Course 2:

☐ Pharmacy
☐ Hospital
☐ STI clinic
☐ Internet
☐ Via a friend or family member
☐ I have used antibiotics which I already had from a previous course
☐ I don't know
☐ Other, namely _____

Course 3:

☐ Pharmacy
☐ Hospital
☐ STI clinic
☐ Internet
☐ Via a friend or family member
☐ I have used antibiotics which I already had from a previous course
☐ I don't know
☐ Other, namely _____

Course 4:

☐ Pharmacy

☐ Hospital

☐ STI clinic

☐ Internet

☐ Via a friend or family member

☐ I have used antibiotics which I already had from a previous course

☐ I don't know

☐ Other, namely _____

f) Please specify for each course of antibiotics you used in the **preceding 6 months** if you finished the course?

Course 1:

☐ Yes

☐ No, because_____

Course 2:

☐ Yes

☐ No, because_____

Course 3:

☐ Yes

☐ No, because_____

Course 4:

☐ Yes

☐ No, because_____

3. The following questions are about the use of antibiotics during your entire life.

a) Have you ever not finished your course of antibiotics?

☐ Yes, regularly

☐ Yes, sometimes

☐ No, never → go to question 10

b) Have you ever saved a course of antibiotics which you did not entirely use for a next time?

☐ Yes, regularly

☐ Yes, sometimes

☐ No, never

Risk factors

4. Have you been abroad for more than 24 hours in the preceding 6 months?
   - ☐ Yes, in the following country/countries:
     1) _____
     2) _____
     3) _____
     4) _____
     5) _____
     6) _____
     7) _____
     8) _____
     9) _____
     10) _____
   - ☐ No

5. Have you been admitted and/or treated in a hospital in the preceding 6 months?
   - ☐ Yes, in the Netherlands
   - ☐ Yes, abroad, in the following country/countries: _____
   - ☐ No

6. Did you eat meat in the preceding 6 months?
   - ☐ Yes, regularly
   - ☐ Yes, sometimes
   - ☐ No, never

7. Please specify if you had one of the following animals in the **preceding 6 months**?
   - ☐ Cat(s)
   - ☐ Dog(s)
   - ☐ Horse(s)
   - ☐ None of the above

8. Did you work in a health care facility in the preceding 6 months, at which you also came into contact with patients?
   - ☐ Yes, in a
     - ☐ Hospital
     - ☐ Nursing home
     - ☐ Hospice
     - ☐ Home care institution
     - ☐ General practice

- ☐ Ambulance service
- ☐ Public health service
- ☐ Other, namely _____

☐ No

9. Did you work for an airline as a pilot or steward in the preceding 6 months?

☐ Yes

☐ No

Supplementary Table 1. Univariable logistic regression analyses of determinants and their association with ESBL-E carriage among 583 MSM participating in the Amsterdam Cohort Studies between April and December 2018, Amsterdam, the Netherlands.

	OR	95% CI	p-value
Demographics			
Age			.166
16-34 years	Ref		
35-44 years	0.60	0.34-1.06	
≥45 years	0.67	0.40-1.11	
Country of birth			.369
The Netherlands	Ref		
Outside the Netherlands	1.30	0.74-2.26	
Education			.055
No college degree	Ref		
College degree or higher	1.71	0.96-3.04	
Known risk factors for AMR			
Antibiotic use in past six months	1.66	1.03-2.68	.041
Travel history in past six months[†]			.436
No travel	Ref		
Travel to low-endemic countries	0.94	0.44-2.02	
Travel to medium-endemic countries	0.72	0.39-1.33	
Travel to high-endemic countries	1.12	0.60-2.06	
Admitted to a hospital in past six months	0.97	0.49-1.93	.932
Potential risk factors for AMR			
HIV status			.660
Negative	Ref		
Positive	0.82	0.34-2.01	
Did ever not finish antibiotic treatment			.791
Yes	Ref		
No, never	1.07	0.50-2.27	
Not applicable (no antibiotic use)	0.71	0.18-2.89	
Saved antibiotics for later[¢]			.108
Yes, occasionally	Ref		
No, never	0.23	0.06-0.93	
Meat consumption			.274
Regularly	Ref		
Sometimes	1.27	0.72-2.20	
Never	1.95	0.84-4.54	
Cat owner	1.27	0.74-2.18	.386
Dog owner	1.42	0.74-2.74	.307

Supplementary Table 1. Univariable logistic regression analyses of determinants and their association with ESBL-E carriage among 583 MSM participating in the Amsterdam Cohort Studies between April and December 2018, Amsterdam, the Netherlands. *(continued)*

	OR	95% CI	p-value
Horse owner	3.15	0.74-13.41	.146
Employed in patient care	0.84	0.40-1.77	.642
Employed as cabin personnel or pilot	2.58	1.13-5.90	.034
Sexual behavior in the past six months			
Number of sexual partners[§]	1.56	1.26-1.93	<.001
Steady partner(s)			
Having a steady partner	0.89	0.55-1.42	.616
Insertive fellatio (receiving blowjob)	1.03	0.64-1.63	.913
Receptive fellatio (giving blowjob)	1.01	0.63-1.61	.971
Insertive anal sex	1.19	0.73-1.95	.486
Receptive anal sex	1.22	0.75-1.98	.430
Rimming partner	1.00	0.60-1.66	.997
Getting rimmed	1.22	0.73-2.02	.453
Casual partner(s)			
Having a casual partner	1.91	1.02-3.58	.032
Insertive fellatio (receiving blowjob)	1.77	1.05-3.01	.028
Receptive fellatio (giving blowjob)	2.35	1.34-4.13	.002
Insertive anal sex	2.02	1.23-3.32	.004
Receptive anal sex	1.89	1.17-3.05	.008
Rimming partner	1.54	0.97-2.46	.067
Getting rimmed	2.04	1.26-3.32	.003
Fisting partner	1.11	0.54-2.28	.733
Getting fisted	1.79	0.81-3.93	.166
Inserting toys in partner	1.45	0.76-2.74	.270
Getting toys inserted	1.32	0.68-2.53	.420
Sharing toys	1.10	0.37-3.32	.863

[†] Low, medium and high endemic countries were based on studies by Arcilla (2016) and Doi (2017).
[ǂ] Among those who ever did not finish their antibiotic treatment.
[§] Included as ln(number of sex partners + 1)
Abbreviations: AMR = antimicrobial resistance; ESBL-E = extended spectrum β-lactamase-producing Enterobacteriaceae; MSM = men who have sex with men; OR = odds ratio; CI = confidence interval; SP = steady partner; CP = casual partner; HIV = human immunodeficiency virus.
Missings: country of birth 4; education 2; not finishing antibiotic treatment 72; saving antibiotics for later 63; number of sex partners 45; having steady partner 35; insertive fallatio with steady partner 35; receptive fallatio with steady partner 35; insertive anal sex with steady partner 36; receptive anal sex with steady partner 36; rimming steady partner 35; getting rimmed by steady partner 35; having casual partner 36; insertive fallatio with casual partner 36; receptive fallatio with casual partner 36; insertive anal sex with casual partner 36; receptive anal sex with casual partner 36; rimming casual partner 36; getting rimmed by casual partner 37; fisting casual partner 39; getting fisted by causal partner 38; inserting toys in casual partner 38; getting toys inserted by casual partner 39; sharing toys with casual partner 90.

Supplementary Table 2. Bayesian Information Criteria (BIC) levels with increasing numbers of classes, k

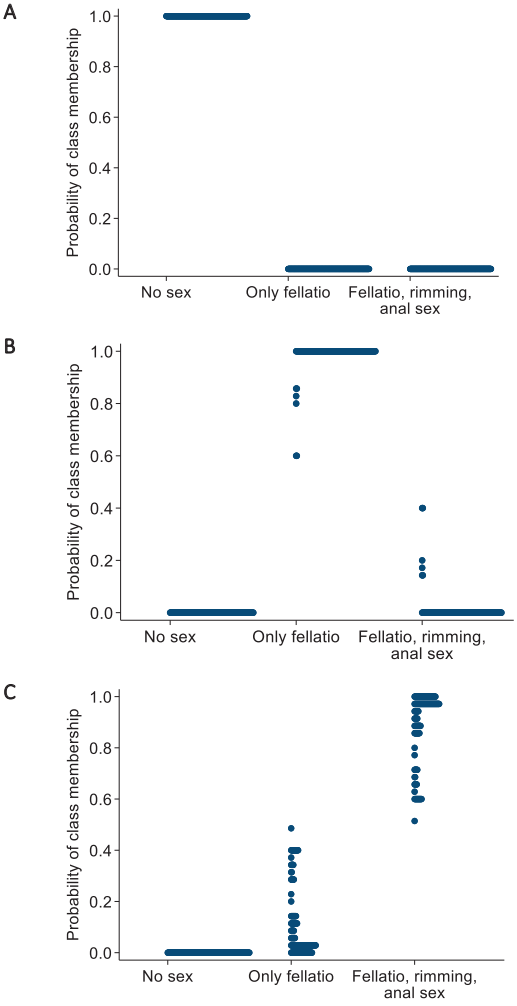
Number of classes (k)	Model on classes of sexual behavior with steady partners	Model on classes of sexual behavior with casual partners
1	4234.8	5991.6
2	2533.5	3967.3
3	2440.1	3811.0
4	No convergence	3732.0
5	No convergence	No convergence

BIC for a latent class model with k clusters of sexual behaviors with steady partners or causal partners. The number of clusters was chosen by the model with the lowest BIC.

Supplementary Table 3. Median number of sexual partners per class of sexual behavior with steady partners and per class of sexual behavior with casual partners.

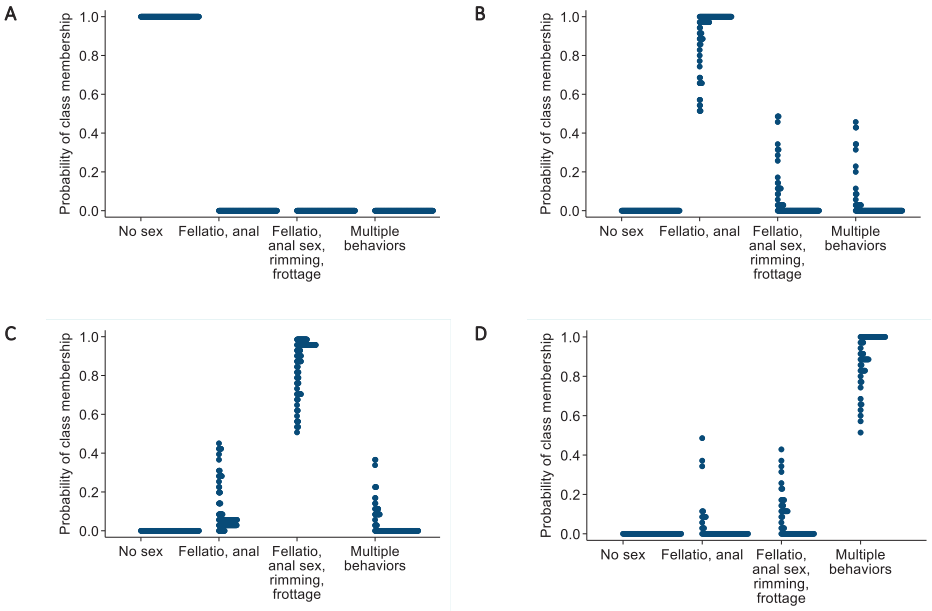
	Number of sexual partners	
	median	[IQR]
Sexual behavior cluster with steady partners*		
(1) no sex with steady partner	6	[3-16]
(2) only fellatio	5	[1-16]
(3) fellatio, rimming and anal sex	4	[1-13]
Sexual behavior cluster with casual partners*		
(1) no sex with casual partner	1	[1-2]
(2) fellatio and anal sex	7	[4-14]
(3) fellatio, anal sex, rimming and frottage	12	[6-26]
(4) multiple behaviors including toy use and fisting	15	[6-26]

*Clusters are defined as latent classes of like sexual behavior, established separately for steady partners and casual partners.



Supplementary Figure 1. Histograms of per class *a posteriori* probabilities of belonging to each class of sexual behavior with steady partners

Three classes of sexual behaviors with steady partners were established. The *a posteriori* probabilities of belonging to each of the three classes, as obtained from the conditional likelihood, are given for individuals who were assigned the "no sex" (**A**), "only fellatio" (**B**), and "fellatio, rimming, anal sex" clusters (**C**).



Supplementary Figure 2. Histograms of per class *a posteriori* probabilities of belonging to each class of sexual behavior with casual partners

Four classes of sexual behaviors with casual partners were established. The *a posteriori* probabilities of belonging to each of the four classes, as obtained from the conditional likelihood, are given for individuals who were assigned the "no sex" (**A**), "fellatio, anal" (**B**), "fellatio, anal sex, rimming, frottage" (**C**), and "multiple behaviors including toy use and fisting" (**D**) clusters.

6

High prevalence of multidrug resistant *Enterobacterales* among residents of long term care facilities in Amsterdam, the Netherlands

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ABSTRACT

Background

The aim of this study was to determine the rate of asymptomatic carriage and spread of multidrug-resistant micro-organisms (MDRO) and to identify risk factors for extended spectrum beta-lactamase-producing *Enterobacteriaceae* (ESBL-E) carriage in 12 long term care facilities (LTCFs) in Amsterdam, the Netherlands.

Methods

From November 2014 to august 2015, feces and nasal swabs from residents from LTCFs in Amsterdam, the Netherlands were collected and analyzed for presence of multidrug-resistant Gram-negative bacteria (MDRGN), including ESBL-E, carbapenemase-producing *Enterobacteriaceae* (CPE), colistin-resistant *Enterobacteriaceae* and methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE). Logistic regression analysis was performed to assess associations between variables and ESBL-carriage.

Results

In total, 385 residents from 12 LTCFs (range 15-48 residents per LTCF) were enrolled. The prevalence of carriage of MDRGN was 18.2% (range among LTCFs 0-47%) and the prevalence of ESBL-E alone was 14.5% (range among LTCFs: 0-34%). Of 63 MDRGN positive residents, 50 (79%) were ESBL-E positive of which 43 (86%) produced CTX-M. Among 44 residents with ESBL-E positive fecal samples of whom data on contact precautions were available at the time of sampling, only 9 (20%) were already known as ESBL-E carriers. The prevalence for carriage of MRSA was 0.8% (range per LTCF: 0-7%) and VRE 0%. One CPE colonized resident was found. All fecal samples tested negative for presence of plasmid mediated resistance for colistin (MCR-1). Typing of isolates by Amplified Fragment Length Polymorphism (AFLP) showed five MDRGN clusters, of which one was found in multiple LTCFs and four were found in single LTCFs, suggesting transmission within and between LTCFs. In multivariate analysis only the presence of MDRO in the preceding year remained a risk factor for ESBL-E carriage.

Conclusions

The ESBL-carriage rate of residents in LTCFs is nearly two times higher than in the general population but varies considerably among LTCFs in Amsterdam, whereas carriage of MRSA and VRE is low. The majority (80%) of ESBL-E positive residents had not been detected by routine culture of clinical specimens at time of sampling. Current infection control practices in LTCFs in Amsterdam do not prevent transmission. Both improvement of basic hygiene, and funding for laboratory screening, should allow LTCFs in Amsterdam to develop standards of care to prevent transmission of ESBL-E.

BACKGROUND

Antimicrobial resistance has been identified as a key public health challenge (1). Amongst the multidrug-resistant micro-organisms (MDRO) are extended spectrum beta-lactamase-producing *Enterobacteriaceae* (ESBL-E), carbapenemase-producing *Enterobacteriaceae* (CPE), vancomycin-resistant enterococci (VRE), and methicillin-resistant *Staphylococcus aureus* (MRSA). The Netherlands is a country with low antibiotic use in humans and is among to the countries with the lowest antibiotic resistance rates in clinical isolates in Europe (2).

Dutch national guidelines for contact precautions for carriers of MDRO (other than MRSA) in Long Term Care Facilities (LTCFs) were published late 2014 (3). In addition to European guidelines for the management of infection control precautions of multidrug-resistant Gram-negative bacteria (MDRGN) in hospitals (4), the Dutch national guidelines also define co-resistance to fluoroquinolones and aminoglycosides in *Enterobacteriaceae* as multidrug resistance. *Pseudomonas aeruginosa* is considered MDRGN when resistance for three out of five of the following antibiotics is detected: carbapenems, aminoglycosides, fluorochinolones, ceftazidim, piperacillin(3).

Previous studies in Amsterdam (2010-2011) showed a prevalence of ESBL-E carriage of 10.6% (95% CI: 9.7–11.5) and 8.6% (95% CI: 7.3-10.0) in patients attending their general practitioner with gastrointestinal symptoms and in the general population, respectively (5, 6). A point prevalence study among 200 patients screened upon admission in a large general hospital in Amsterdam in 2014 (7), showed a MDRGN prevalence of 10.5%, of which 76% was identified as ESBL-E.

Outbreaks of MDRGN are rarely detected and only incidentally reported in Dutch LTCFs (8). Point prevalence studies in Dutch LTCFS have shown a large variation in MDRO carriage rates, ranging from 4% to 21% (8-12). The role of LTCFs in the transmission of MDRO within the Dutch healthcare network and interventions needed to prevent transmission of MDRO in LTCFs are still under debate (13, 14). Our aim was to study the prevalence, risk factors and molecular epidemiology of carriage of MDRO among residents of LTCFs in Amsterdam.

METHODS

Setting and Data Collection

For this cross-sectional study we made a selection of LTCFs in Amsterdam that provided assisted-living and intensive nursing and harbored at least 50 residents. LTCFs with different types of nursing wards (psychogeriatric, somatic, rehabilitation, or a combination of these wards) were included to obtain an equal number of patients of each type of ward. Resident-related risk factors for carriage of MDRO were assessed by a questionnaire that was completed by LTCF nursing staff. Institutional risk factors were assessed through a questionnaire that was completed by the LTCF management staff and during a site visit at participating LTCF wards by an expert in infection control. Risk factors were scored, using scoring lists adapted from a previously validated infection risk scan (IRIS) (12).

Sample collection

Nasal swabs (Copan eMRSA™, Brescia, Italy) and feces (COPAN FecalSwab™, Brescia, Italy) were collected from each participating resident by local nursing staff.

MDRGN definition

MDRGN were defined as used by the Dutch national guidelines. *Enterobacteriaceae* were considered MDRGN when they were ESBL or carbapenemase-producing or if they harbored a co-resistance

Laboratory detection

After overnight incubation (37°C), nasal swabs were cultured on chromID™ MRSA agar (bioMerieux, Marcy l'Etoile, France). Feces was cultured on chromID™ MRSA agar after overnight incubation in nutrient broth no.2 + 6% NaCl (Media Products, Groningen, the Netherlands). Feces was additionally screened for 1) multidrug-resistant Gram-negative organisms using overnight incubation of an amoxicillin (16mg/L) containing BHI broth (Media Products) subcultured to MacConkey agar plates (Media Products) with cefotaxime (5ug) and ceftazidim (10 ug) neo-sensitabs (Rosco Diagnostica, Taastrup, Denmark) and MacConkey agar plates containing 16 ug/L gentamicin with a ciprofloxacin neo-sensitab (10ug) and 2) VRE using overnight incubation of an antibiotic free Enterococcosel™ enrichment broth (Becton Dickinson, Utrecht, Netherlands) and chromID™ VRE (bioMerieux) agar plates. Identification and antimicrobial susceptibility testing (N200 card) of isolates was performed by standard methods and phenotypic confirmation of ESBL by E-test in accordance with EUCAST (15) and Dutch national guidelines (16). Confirmation and genotyping of MRSA and CPE was performed by the Dutch reference laboratory at the National Institute for Public Health and the Environment (RIVM). Amplified fragment

length polymorphism (AFLP) was performed on all available multidrug-resistant *Enterobacteriaceae* isolates as described in the supplementary methods. Isolates were considered indistinguishable (representing a cluster) when the band patterns were >90% identical. For phylogenetic typing, a selection of *Escherichia coli* isolates were further analyzed by phylogroup-defining PCR (17). Group B2 *E. coli* were further characterized by O25:ST131-specific PCR (18). All phenotypically ESBL-positive isolates were tested for the presence of CTX-M, SHV and TEM ESBL resistance genes by PCR as previously described (19, 20). The CTX-M, SHV and TEM ESBL resistance genes were additionally typed by sequencing. Sequencing was performed as described in the supplementary methods. Primer sequences are listed in Supplementary Table 1. Consensus sequences were uploaded at The Comprehensive Antibiotic Resistance Database BLAST service for typing (Jia et al., at http://arpcard.mcmaster.ca) (21). The MCR-1 PCR was performed by the Department of Medical Microbiology of Leiden University Medical Centre according to methods described previously by Nijhuis et al. (22) and Terveer et al. (23). All laboratory detection methods are described in the supplementary methods.

Statistical analysis

Associations between variables and ESBL carriage were assessed by univariable logistic regression analysis. All variables with an associated $p<0.25$ in univariable analyses were included in a multivariable model, with the exception of type of room and use of contact precautions at the time of sampling (since these factors might be a consequence of previously detected ESBL-carriage). A backwards-stepwise procedure was performed by sequentially removing any variable with a p-value >0.05 in order to obtain a final multivariable model. Results are presented as odds ratios (OR) with corresponding 95% confidence intervals (CI). Confidence intervals that did not contain 1 were considered statistically significant. All statistical analyses were performed with SPSS version 21.0 (SPSS Inc., Chicago, IL, USA).

Ethical considerations

This study was reviewed and approved by the Medical Ethical Committee of the VU Medical Center Amsterdam (protocol ID NL50241.018.14). The study was judged to be beyond the scope of the Medical Research Involving Human Subjects Act (in Dutch, Wet Medisch-wetenschappelijk Onderzoek met Mensen [WMO]), and a waiver of written informed consent was obtained. Patients who participated in the study provided verbal informed consent for use of demographic, clinical, and culture data.

RESULTS

Participating LTCFs and residents

Twenty-four Amsterdam LTCFs were approached of whom ten participated in this study. Because of a lower response rate than expected we additionally approached one LTCF in Zaandam (LTCF L, 15km from Amsterdam) and one LTCF post acute care ward located in a large teaching hospital (<50 residents, LTCF K). The main reason for non-participation was the expected workload of sampling. Characteristics of the participating LTCFs are listed in Table 1. From November 2014 to August 2015, 385 residents from 12 LTCFs (range 15-48 residents per LTCF, 1730 residents in total) were enrolled. For 30 residents sample collection was either not or inadequately performed and they were excluded from further analysis. For another 36 residents the questionnaire was missing. For 310 residents both a fecal swab and questionnaire were available for analysis for MDRO colonization. Key participant characteristics of residents with both a fecal swab and questionnaire available for analysis are summarized in Table 2.

Table 1. Characteristics and MDRGN prevalence of 12 participating LTCFs

LTCF	No. of residents	No. of samples	ESBL+ residents		Total MDRGN#		MDRGN cluster analysis by AFLP
			N	%	N	%	
A	125*	34	6	17.6%	7	20.6%	Cluster 1 (5 residents), Cluster 5
B	130	34	6	17.6%	7	20.6%	Cluster 1 (2 residents), Cluster 4
C	193	42	2	4.8%	5	11.9%	
D	189	17	0	0.0%	0	0.0%	
E	108	17	0	0.0%	0	0.0%	
F	144	32	11	34.4%	15	46.9%	Cluster 1 (4 residents), Cluster 2, Cluster 3
G	110	33	6	18.2%	7	21.2%	
H	199	39	6	15.4%	8	20.5%	Cluster 1 (1 resident)
I	144	32	9	28.1%	9	28.1%	
J	96	18	1	5.6%	2	11.1%	
K	20	13	3	23.1%	3	23.1%	
L	272	35	0	0.0%	0	0.0%	
Total	**1,730**	**346**	**50**	**14.5%**	**63**	**18.2%**	

Includes ESBLE-E, Carbapenemase-producing *Enterobacteriaceae*, Multidrug-resistant *P. aeruginosa* and aminoglycoside-fluoroquinolones co-resistant *Enterobacteriaceae*

* Estimated number based on historical data

Abbreviations: MDRGN = Multidrug-resistant Gram-negative bacteria; LTCF = Long term care facility; No. = number; ESBL-E = Extended Spectrum Beta Lactamase-producing *Enterobacteriaceae*; AFLP = Amplified Fragment Length Polymorphism

Table 2. Demographics and clinical characteristics of participating residents. Cases are ESBL-E carriers. Univariable associations of demographic and clinical characteristics with ESBL carriage of LTCF participants with both a fecal swab and questionnaire available for analysis (N=310).

Variable	Cases*/Total		OR	95%CI	p-value
	N	%			
Sex					.590
Female	30/199	15.1%	Ref		
Male	14/109	12.8%	0.83	0.42-1.64	
Age					.168
<70 years	2/39	5.1%	Ref		
70-79 years	11/61	18.0%	4.07	0.85-19.47	
80-89 years	22/129	17.1%	3.80	0.85-16.96	
≥90 years	9/73	12.3%	2.60	0.53-12.69	
Nursing indication					.689
Psychogeriatric	14/108	13.0%	Ref		
Somatic	19/137	13.9%	1.08	0.51-2.27	
Rehabilitation	11/62	17.7%	1.45	0.61-3.42	
Antimicrobial use in previous 30 days					.899
No	39/273	14.3%	Ref		
Yes	5/37	13.5%	0.94	0.34-2.55	
Current antimicrobial use					.888
No	43/302	14.2%	Ref		
Yes	1/8	12.5%	0.86	0.10-7.17	
Hospitalization in previous 90 days					.449
No	33/218	15.1%	Ref		
Yes	9/77	11.7%	0.74	0.34-1.63	
MDRO detected in previous year					<.001
No	35/289	12.1%	Ref		
Yes	9/15	60.0%	10.89	3.65-32.43	
Type of room					
Single person	32/201	15.9%	#	#	
Multiple person	9/88	10.2%	#	#	
Contact precautions at time of sampling					
No	35/293	12.0%	#	#	
Yes	9/14	64.3%	#	#	

Table 2. Demographics and clinical characteristics of participating residents. Cases are ESBL-E carriers. Univariable associations of demographic and clinical characteristics with ESBL carriage of LTCF participants with both a fecal swab and questionnaire available for analysis (N=310). *(continued)*

Variable	Cases*/Total		OR	95%CI	p-value
	N	%			
Length of stay					.401
0-10 weeks	13/73	17.8%	Ref		
11-64 weeks	7/74	9.5%	0.48	0.18-1.29	
65-161 weeks	10/73	13.7%	0.73	0.30-1.80	
162-670 weeks	13/73	17.8%	1.00	0.43-2.33	
Decubitus wounds					.796
No	41/285	14.4%	Ref		
Yes	3/24	12.5%	0.85	0.24-2.98	
Other wounds					.534
No	39/279	14.0%	Ref		
Yes	5/27	18.5%	1.40	0.50-3.91	
Pneumonia in medical history					.508
No	34/251	13.6%	Ref		
Yes	10/59	16.9%	1.30	0.60-2.81	
Comorbidities					
Diabetes					.097
No	29/236	12.3%	Ref		
Yes	15/74	20.3%	1.81	0.92-3.61	
COPD					.050
No	34/270	12.6%	Ref		
Yes	10/40	25.0%	2.31	1.04-5.15	
Vascular disorder					.819
No	21/143	14.7%	Ref		
Yes	23/167	13.8%	0.93	0.49-1.76	
Renal impairment					.893
No	33/235	14.0%	Ref		
Yes	11/75	14.7%	1.05	0.50-2.20	
IBD					
No	44/305	14.4%	-	-	
Yes	0/5	0.0%	-	-	

Table 2. Demographics and clinical characteristics of participating residents. Cases are ESBL-E carriers. Univariable associations of demographic and clinical characteristics with ESBL carriage of LTCF participants with both a fecal swab and questionnaire available for analysis (N=310). *(continued)*

Variable	Cases*/Total		OR	95%CI	p-value
	N	%			
Other					
No	43/307	14.0%	Ref		
Yes	1/3	33.3%	3.07	0.27-34.59	.401
Current infections					
Sepsis/bacteremia					
No	44/310	14.2%	-	-	
Yes	0/0	-	-	-	
Urinary tract infection					.901
No	42/297	14.1%	Ref		
Yes	2/13	15.4%	1.10	0.24-5.16	
Upper respiratory tract infection					.153
No	42/305	13.8%	Ref		
Yes	2/5	40.0%	4.17	0.68-25.73	
Lower respiratory tract infection					.994
No	43/303	14.2%	Ref		
Yes	1/7	14.3%	1.01	0.12-8.58	
Gastro-intestinal tract infection					
No	44/307	14.3%	-	-	
Yes	0/3	0.0%	-	-	
Skin infection					.994
No	43/303	14.2%	Ref		
Yes	1/7	14.3%	1.01	0.12-8.58	
Medical devices					
Urinary catheter					.361
No	42/286	14.7%	Ref		
Yes	2/24	8.3%	0.53	0.12-2.33	
Suprabubic catheter					
No	44/303	14.5%	-	-	
Yes	0/7	0.0%	-	-	

Table 2. Demographics and clinical characteristics of participating residents. Cases are ESBL-E carriers. Univariable associations of demographic and clinical characteristics with ESBL carriage of LTCF participants with both a fecal swab and questionnaire available for analysis (N=310). *(continued)*

Variable	Cases*/Total		OR	95%CI	p-value
	N	%			
PEG tube					.721
No	43/305	14.1%	Ref		
Yes	1/5	20.0%	1.52	0.17-13.95	
Vacuum therapy					
No	43/294	14.6%	-	-	
Yes	0/0	-	-	-	
Intravascular catheter					
No	44/309	14.2%	-	-	
Yes	0/1	0.0%	-	-	
Incontinence					
Urine					.672
No	19/143	13.3%	Ref		
Yes	25/167	15.0%	1.14	0.60-2.19	
Feces					.926
No	22/157	14.0%	Ref		
Yes	22/153	14.4%	1.03	0.54-1.95	

* Cases are defined as carriers of ESBL
# Not estimated since contact measures at time of sampling and staying in a single vs. multiple person room might be a consequence of known ESBL-E carriage
Missings: sex 2; age 8; nursing indication 3; decubitis wounds 1; other wounds 4; hospitalization in previous 90 days 15; MDRO detected in previous year 6; type of room 21; ICP at time of sampling 3; length of stay 17; vacuum therapy 16
Abbreviations: CI = Confidence interval; OR= Odds ratio; IQR = Inter quartile range; COPD = Chronic Obstructive Pulmonary Disease; IBD = Inflammatory Bowel Disease; PEG = Percutaneous Endogastric; MDRO = Multidrug-resistant micro-organisms; ESBL = Extended Spectrum Beta Lactamase

Prevalence of carriage of MDRO

The prevalence of carriage of MDRGN was 18.2% (range among LTCFs 0-47%) and the prevalence of ESBL-E alone was 14.5% (range among LTCFs: 0-34%) (Table 1). The prevalence of carriage of MRSA was 0.8% (range per LTCF: 0-7%) and of VRE 0%. The three carriers of MRSA resided in three different LTCFs and did not carry MDRGN. In total, 71 unique MDRGN isolates were cultured from 63 residents; 53/71 (75%) isolates from 50 residents phenotypically produced ESBL, of which 39 (74%) were identified as *E. coli*, 12 (23%) as *Klebsiella pneumoniae* and two as *Enterobacter cloacae* and *Citrobacter freundii*. Thirteen ESBL-producing isolates were co-resistant to fluoroquinolones and aminoglycosides and one *K. pneumoniae* isolate also produced New Delhi Metallo-beta-

lactamase-1 (NDM). The prevalence of ESBL-E carriage among LTCF residents was 14.5% (95% CI: 10.8–18.2). Of the remaining 18 non-ESBL isolates, 17 *Enterobacteriaceae* were resistant to the combination of aminoglycosides and fluoroquinolones and one isolate identified as *P. aeruginosa* additionally resistant to piperacillin. All 346 fecal samples were negative for presence of the MCR-1 gene.

Molecular characterization and ESBL typing

A total of 7/71 (10%) MDRGN isolates had not been stored and could not be retrieved from the small quantities of original sample kept by -80 degrees Celsius. The missing isolates have a similar distribution of species identification, resistance pattern and LTCF location as the selection used for molecular analysis. The presence of genes encoding ESBL was confirmed in all phenotypically ESBL-producing isolates except for one isolate which had a TEM-1 gene only (no ESBL). However, another *E. coli* isolate of the same resident with a different AFLP-result was genotypically confirmed as ESBL. The ESBL-genes most frequently detected were CTX-M-15 (16/51, 31%) and CTX-M-27 (12/51, 24%) (Table 3). In total, 5 clusters varying in size from 2 to 12 strains, were detected in 4 LTCFs by phylogenetic analysis of AFLP-results (Table 1). Figure 1 depicts AFLP-results of all *E. coli* isolates with one representative isolate per cluster. All *E. coli* isolates from clusters

Table 3. ESBL-encoding genes (1 sequence per cluster, see Methods)

ESBL family	ESBL gene/type	N
CTX-M-1 family	*bla*$_{\text{CTX-M-15}}$	16*
	bla$_{\text{CTX-M-1}}$	4
CTX-M-9 family	*bla*$_{\text{CTX-M-14}}$ *bla*$_{\text{CTX-M-14 /17\$}}$	7 1
	bla$_{\text{CTX-M-9}}$	2
	bla$_{\text{CTX-M-27}}$	12
CTX-M#	*bla*$_{\text{CTX-M}}$	1
TEM and SHV+	*bla*$_{\text{TEM-52}}$	3
	bla$_{\text{TEM-20}}$	1
	bla$_{\text{SHV-2}}$	1
	bla$_{\text{SHV-12}}$	2
Total		**50**

* One isolate also encoded New Delhi Metallo-beta-lactamase-1
$ No discrimination between CTX-M-14 and CTX-M-17. One strain was phenotypically ESBL, but no ESBL gene could be detected.
# Exact subtype of one CTX-M gene remained unresolved by sequencing
+ Possibly, there were more TEM or SHV ESBL genes present. TEM or SHV was not sequenced from CTX-M-positive strains.
Abbreviations: N = number; ESBL = Extended Spectrum Beta Lactamase

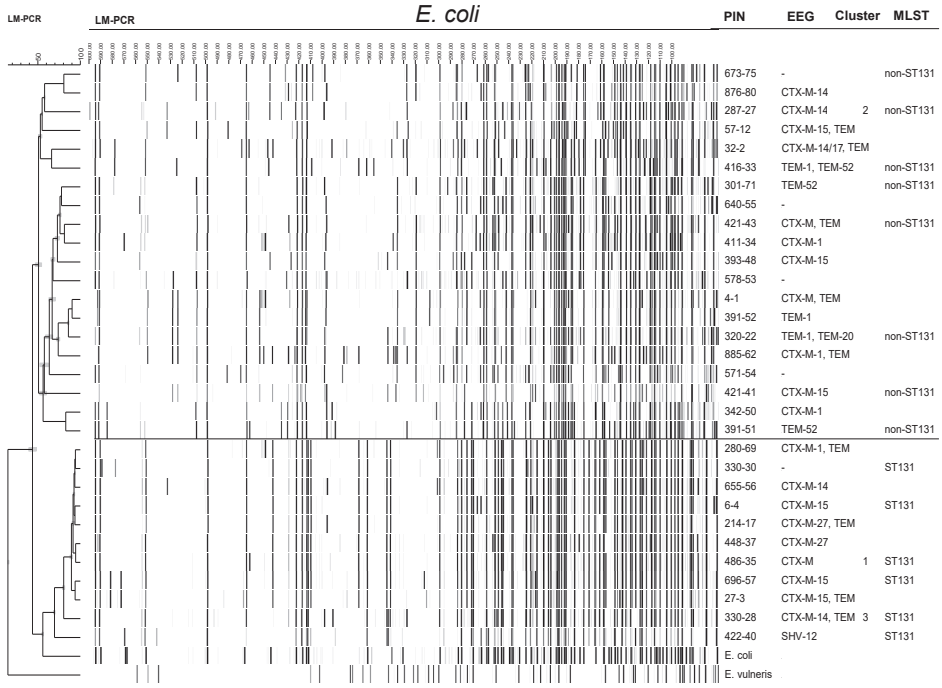

Figure 1. AFLP results of all *E. coli* isolates with one representative isolate per cluster.
Abbreviations: PIN = patient identification number; EEG = ESBL encoding gene; P-PCR = phylogroup defining polymerase chain reaction

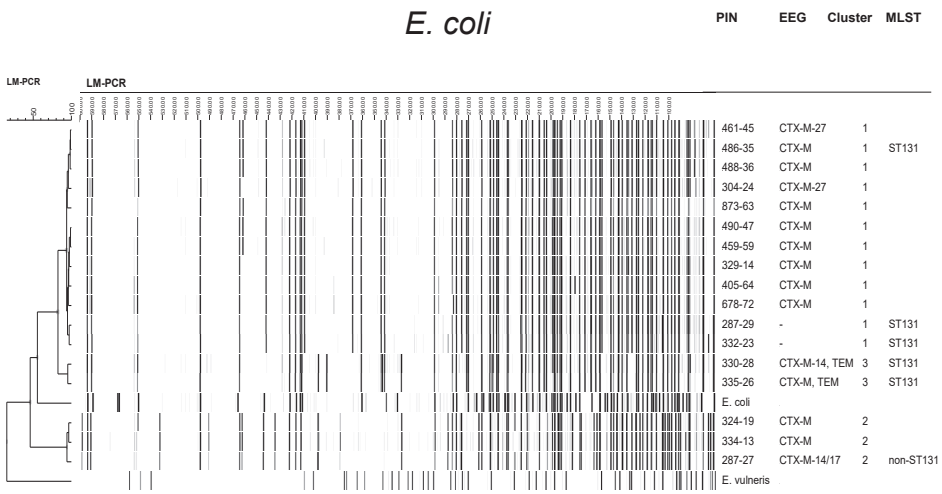

Figure 2. *E. coli* isolates from clusters 1-3.
Abbreviations: PIN = patient identification number; EEG = ESBL encoding gene; P-PCR = phylogroup defining polymerase chain reaction

1-3 are depicted in Figure 2. A total of 22/63 (35%) MDRGN carriers from our study could be clustered with at least one other MDRGN carrier.

Phylogenetic typing was performed on a selection of 19 *E. coli* isolates, dividing the phylogenetic tree in half with 22/45 (49%) non-ST131 *E. coli* isolates and 23/45 (51%) ST131 *E. coli* isolates (Figure 1). Isolates from clusters 1 and 3 belong to the ST131 genotype.

Risk factors for carriage of ESBL

For 310 of 385 residents both results from fecal sample cultures and from questionnaire were available; these were used for analysis of resident-related risk factors for carriage of ESBL-E. Among 44 residents with ESBL-E positive fecal samples of whom data on contact precautions were available at the time of sampling, only 9 (20%) were already known as ESBL-E carriers. In the univariable logistic regression analysis the following risk factors ($p<0.25$) were associated with ESBL-carriage: age, MDRO carriage in the preceding year, diabetes mellitus, COPD and having a current upper respiratory tract infection (Table 2). In the multivariable logistic regression analyses only the presence of a MDRO in the preceding year remained a risk factor for ESBL-carriage (OR 10.9, 95%CI: 3.7-32.4).

DISCUSSION

The present study showed that nearly one in five residents carried MDRGN in LTCFs in Amsterdam. Phylogenetic analysis showed five clusters of isolates in four LTCFs, suggesting transmission of ESBL-E within and between LTCFs. The large majority of MDRGN were ESBL-E, with a prevalence of carriage of nearly one in seven residents. The prevalence of MRSA was less than 1%, while no carriers of VRE were found.

Our study suggests a higher prevalence of ESBL-E carriage in nursing home residents than in the general population in the Amsterdam area. The majority of these carriers was only detected during the prevalence survey, hence, most carriers remain undetected.

Verhoef et al (9) found an overall prevalence of ESBL-resistance genes of 4.2% in *E. coli* isolates isolated from urine samples in 107 Dutch LTCFs in 2012-2014. Only one LTCF from Amsterdam participated in that survey, and no cases of ESBL-E were detected among its residents. This prevalence is likely to be an underestimation because Verhoef only focused on ESBL-producing *E. coli* in urine samples, and not on gastrointestinal carriage of ESBL-E. LTCFs in the Amsterdam area are underrepresented in national surveillance studies such as SNIV (surveillance network in LTCFs), hampering actual insight and control plans for MDRO. However, healthcare inspectorate reports suggest that quality and

safety of care in LTCFs in Amsterdam are compromised more often compared to acute care facilities (9, 24).

Preliminary results of a recent national surveillance point-prevalence study for intestinal carriage of resistant bacteria show an ESBL-E prevalence of 9.5% (range 0-22%) in eight nursing homes where feces samples were collected from 337/448 (75%) of residents (10). In other Dutch studies, fecal ESBL-carriage was demonstrated in 70/643 (10.9%) nursing home residents (12) and in 50/579 (8.6%) residents of nursing homes screened upon hospital admission compared to 61/772 (7.9%) elderly who still lived in their own homes (11). A study performed in region Leiden revealed fecal ESBL-carriage of 11% (E.M. Terveer and E.J. Kuijper, manuscript submitted).

The ESBL-E prevalence in our study was significantly higher than that of nearly 9% found in the general population in Amsterdam in 2011 (6). In that study, age was not associated with a higher risk of ESBL-E carriage. Although the prevalence of ESBL-E may have increased in the general population since 2011, our study indicates that LTCFs in Amsterdam may represent a potential reservoir for MDRO in the healthcare network.

The majority of ESBL-E carriers was not detected by routine culture of clinical specimens and were only detected during the prevalence survey. The high proportion of ESBL-E carriers that were additionally detected in our study, may be explained by the restrictive diagnostic policy in LTCFs, and the absence of surveillance. Applying additional contact precautions only to the few known carriers of ESBL-E will very likely result in on-going transmission among residents and to other healthcare institutions. The current infection control policy, which does not include surveillance or regular screening, is likely to be ineffective.

The ESBL-E carriage prevalence ranged from 0% to 34% between participating LTCFs in our study. In a previous survey of a single LTCF in the South of the Netherlands, ESBL-E carriage rates varied substantially between wards, between 0% and 47% (8). This means that the outcome of a single survey is highly dependent on the selection of wards in the LTCF. This also indicates that good quality prognostic determinants of ESBL-E transmission in LTCFs are needed.

The distribution of ESBL-encoding genes in our study is similar to that in the general population of Amsterdam (6) with the exception of CTX-M-27, which was more prevalent in nursing homes. This, however may be related to the presence of a cluster of isolates with this gene (cluster 1). While nearly 16% of ESBL-E in the general population of Amsterdam belong to the ST131 MLST genotype, in LTCFs, nearly 50% of ESBL-E belong to

this easily expanding, more virulent and better persisting genotype (25, 26). The only cluster of isolates that extended over more than one LTCF in our study belonged to ST131. The overrepresentation of ST131 in LTCFs could be due to clonal expansion since the study performed in the general population, or may be due to a higher transmission rate of ESBL-E (or exposure to a common source) in LTCFs. ST131 clone is associated with community-acquired infections and older age and is frequently observed in nursing homes throughout Europe (27).

In our study, one third of MDRGN isolates could be clustered with at least one other MDRGN isolate, suggesting a high transmission rate of MDRGN. A similar high rate (54%) was found in two geriatric rehabilitation wards in Israel (28). In a recent study, Kluytmans-van den Berg et al. analyzed 2005 ESBL-E isolates from 690 ward-based prevalence surveys performed in 14 Dutch hospitals over a period of three years. With core genome Multilocus Sequence Typing (cgMLST) they showed a clonal relation between 2.3% of the isolates at ward level, 1.0% at institution level and 0.5% between institutions (29). This finding suggests that in Dutch hospitals the transmission rate of ESBL-E between patients is low, which was also found in Swiss hospitals (30, 31). Our findings, however, indicate that ESBL-E transmission within LTFCs might be higher.

Our study has some limitations. More than half of the initially selected LTCFs refused to participate, mainly because of time constraints. The LTCFs that did participate endorsed the importance of a point prevalence survey, and of infection control. This selection bias may have resulted in an underestimation of the MDRO prevalence.

Due to the low participation rate of residents within participating LTCFs (<20% in some LTCFs), it is not possible to make robust statements concerning transmission. Furthermore, in our study we could not associate current carriage of ESBL with known risk factors described in literature (32), except for being diagnosed with a MDRO in the preceding year. This could be due to the relative small sample size.

In conclusion, our data show that the carriage rate of ESBL-E in Amsterdam is significantly higher in LTCFs than in the general population, and varies considerably between LTCFs. The prevalence of MRSA and VRE, on the contrary, is low. No MCR-1 colistin-resistance was detected in the MDRGN isolates. Resistance due to the expansion of CTX-M ESBLs, in particular CTX-M-15, is emerging in LTCFs in Amsterdam. About half of multidrug-resistant *E. coli* appear to be related to the international clonal complex ST131. The majority of ESBL-E carriers are undetected in LTCFs in Amsterdam and current infection control practices do not prevent transmission. Both improvement of basic hygiene, and fund-

ing for laboratory screening, should allow LTCFs in Amsterdam to develop standards of care to prevent transmission of ESBL-E.

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Conflicts of interest

None declared.

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SUPPLEMENTARY MATERIAL

Supplementary methods

Laboratory detection

After overnight incubation (37°C), nasal swabs (Copan eMRSA™, Brescia, Italy) were cultured on chromID™ MRSA agar (bioMerieux, Marcy l'Etoile, France). Feces (COPAN FecalSwab™, Brescia, Italy) was cultured on chromID™ MRSA agar after overnight incubation in nutrient broth no.2 + 6% NaCl (Media Products, Groningen, the Netherlands). Feces was additionally screened for 1) multidrug-resistant Gram-negative organisms using overnight incubation of an amoxicillin (16mg/L) containing BHI broth (Media Products) subcultured to MacConkey agar plates (Media Products) with cefotaxime (5ug) and ceftazidim (10 ug) neo-sensitabs (Rosco Diagnostica, Taastrup, Denmark) and MacConkey agar plates containing 16 ug/L gentamicin with a ciprofloxacin neo-sensitab (10ug) and 2) vancomycin-resistant enterococci (VRE) using overnight incubation of an antibiotic free Enterococcosel™ enrichment broth (Becton Dickinson, Utrecht, Netherlands) and chromID™ VRE (bioMerieux) agar plates. Identification and antimicrobial susceptibility testing (N200 card) of isolates was performed by standard methods using the Vitek2 instrument (bioMérieux) and phenotypic confirmation of ESBL by E-test in accordance with EUCAST (1) and Dutch national guidelines (2). Confirmation and genotyping of MRSA and CPE was performed by the Dutch reference laboratory at the National Institute for Public Health and the Environment (RIVM).

Amplified fragment length polymorphism (AFLP)

AFLP was performed mostly as described (3). Briefly, bacterial cells were lysed with Tris-EDTA-buffer (10 mM Tris-HCL, 1 mM EDTA). The lysate was centrifuged, and the supernatant was used for AFLP. The restriction/ligation reaction mixtures consisted of approximately 10 ng DNA, 1x T4 DNA ligase buffer, 0.05 M NaCl, 1 µg BSA, 2 pmol of the EcoRI adapter, 20 pmol of the MseI adapter (Eurogentec, Maastricht, the Netherlands), 160 U of T4 DNA ligase, 2 U of EcoRI and 2 U of MseI. All enzymes were purchased from New England Biolabs (Leiden, Netherlands). After incubation at 37°C for 1 h, the mixtures were diluted 1:20 in water. 5 µl of the mixture was added to 5 µl of PCR mixture, which consisted of 1x PCR buffer (Sphaero Q, Gorinchem, The Netherlands), 2,5 mM MgCl, 350 µM dNTPs (Promega, Leiden, Netherlands), 1 U Super Taq Plus polymerase (Sphaero Q), and 20 ng of Eco-A primer and 60 ng of Mse-C primer (4). The Eco-A primer was fluorescently labelled with carboxyfluorescein (Eurogentec). Amplification was carried out under the following conditions: 2 min at 72°C, followed by 12 cycles of 30 s at 94°C, 30 s starting at 65°C and gradually reduced by 0.7 °C per cycle, and 1 min at 72°C, and then 23 cycles of 30 s at 94°C, 30 s at 56°C and 1 min at 72°C and ended by a single extension at

72°C for 10 min. 2.5 µl of each PCR product was added to 22 µl Hi-Di formamide and 0.5 µl GeneScan-600 LIZ size standard (Applied Biosystems, Bleiswijk, Netherlands). Fragments were separated on an ABI Prism 3130 sequencer (Applied Biosystems). Data were analysed with the GENESCAN analysis software (Applied Biosystems) and BioNumerics software package, version 6.6 (Applied Maths, Sint-Martens-Latem, Belgium). Similarity coefficients were calculated with Pearson correlation and dendrograms were obtained by the unweighted pair group method using arithmetic averages (UPGMA) clustering. The analysis was performed for fragments with lengths between 60 and 600 bp. Isolates were considered indistinguishable (representing a cluster) when the band patterns were identical.

Phylogenetic typing

A selection of *E. coli* strains was subjected by phylogroup-defining PCR (5). Group B2 *E. coli* underwent O25:ST131-specific PCR (6).

Detection of resistance genes

The presence of CTX-M, SHV and TEM ESBL genes was confirmed by High Resolution or SYBR Green melting curve analysis PCR of cell lysates from all phenotypically confirmed ESBL-positive strains. (CTX-M primer sequences in Mulvey et al. 2003 (7), TEM and SHV primer sequences in Agerso et al. 2012 (8)). The PCR contained Precision Melt Supermix (CTX-M PCR) or SYBR Green Supermix (SHV and TEM PCR) buffers (Bio-Rad, Veenendaal, Netherlands), 150 nM of each primer, and bacterial lysate. Amplification was carried out in a LightCycler 480 instrument (Roche, Almere, The Netherlands) under the following conditions: 10 min at 95°C, followed by 35 cycles of 30 s at 95°C, 30 s at 55 °C and 45 s at 72°C. Hereafter 1 min at 95°C and 1 min at 40 °C. A melting curve was recorded by heating between 65°C and 95° with a ramp rate of 0.02 °C/s. Melting curves were converted to melting peaks by the LightCycler software. Distinct peaks were registered for CTX-M groups 1, 2, 8 and 9.

Fecal samples were subjected to MCR-1 PCR by the Leiden University Medical Centre according to methods described previously by Nijhuis et al. (9) and Terveer et al. (10).

Molecular typing of ESBL genes

The SHV, TEM and CTX-M genes were typed by sequencing. The TEM products obtained above were directly used for sequencing with the PCR primers and primers TEM-F2 and TEM-R2. The SHV genes were amplified with primers SHV-F1 and SHV-R1 and sequenced with amplification primers and primers SHV-F2 and SHV-R2. CTX-M groups 1 and 9 were amplified with primers CTX-M-1F and CTX-M-1R, and CTX-M-9F and CTX-M-9R, respectively. Sequencing was performed with amplification primers and the PCR primers used

for detection of resistance genes. Amplification protocol was as above (detection of resistance genes). Primer sequences are listed in Supplementary Table 1. All PCR products were purified with the QIAquick PCR purification kit according to the instructions of the manufacturer (Qiagen, Venlo, Netherlands). Sanger sequencing was performed with the BigDye Terinator v3.1 Cycle Sequencing Kit (Applied Biosystems, Bleiswijk, Netherlands) with 2 pmol/ µl primer. Sequence conditions were 1 min at 96°C, followed by 25 cycles of 10 s at 96°C, 5 s at 55 °C and 4 min at 60°C, ending at 4°C. Sequence products were precipitated with isopropanol and dissolved in formamide, after which fragments were separated on an ABI Prism 3130 sequencer (Applied Biosystems). Sequences were analysed with the CodonCode Aligner software (CodonCode Corporation, Centerville, USA). Consensus sequences were uploaded at The Comprehensive Antibiotic Resistance Database BLAST service for typing (Jia et al., at http://arpcard.mcmaster.ca). (11)

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Supplementary table 1. Primer sequences

Oligonucleotide	Sequence 5'-3'	Reference*
SHV-F1	CTTTACTCGCCTTTATCG	1.
SHV-R1	TTAGCGTTGCCAGTGCTC	2.
SHV-F2	ACTGCCTTTTTGCGCGAGAT	1.
SHV-R2	CAGTTCCGTTTCCCAGCCGT	1.
CTX-M-1F	ATGGTTAAAAAATCACTGCG	3. (CTX-M-10-1F)
CTX-M-1R	CCGTTTCCGCTATTACAAAC	4. (preCTX-M)
CTX-M-9F	TGGTGACAAAGAGAGTGCAACG	3. (CTX-M-9-1F)
CTX-M-9R	TCCTTCAACTCAGCAAAAGT	5. (CTX-M-9-AS)
TEM-F2	TAACCATGAGTGATAACACT	1.
TEM-R2	CCGATCGTTGTCAGAAGTAA	1.

* Original primer names between brackets

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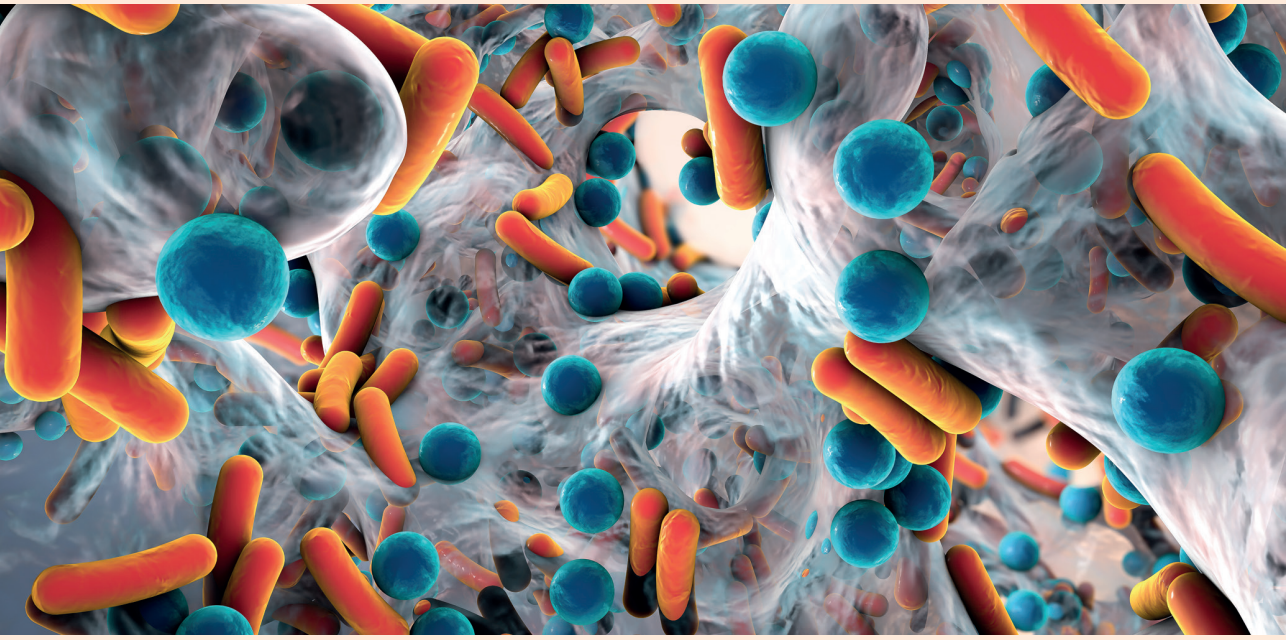

Part III

Healthcare providers' perceived barriers and enablers for preventing the spread of antimicrobial resistance during patient transfers

7

Perceived barriers and enablers for preventing the spread of carbapenemase producing gram-negative bacteria during patient transfers: a mixed methods study among healthcare providers

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ABSTRACT

Background

Antimicrobial resistance (AMR) increasingly threatens public health. Carbapenemase-producing gram-negative bacteria (CPB) pose the biggest threat. The risk for CPB spread is heightened during the transfer of a CPB-positive patient between different healthcare institutions or healthcare providers. We aimed to gain insight into the frequency of CPB-positive patients in the Dutch provinces of Noord-Holland (NH) and Flevoland (FL). Secondly, we aimed to obtain a deeper understanding of the communication between healthcare providers during transfers of CPB-positive patients and explore possible communication-related risk situations for CPB spread.

Methods

This mixed-methods study consisted of a quantitative and qualitative section. For the quantitative section, 14 laboratories that provide diagnostics in NH and FL voluntarily reported carbapenemase-producing *Enterobacteriaceae* (CPE) positive patients between February 2018 and February 2019. Additionally, two laboratories reported carbapenem-resistant *Acinetobacter spp.* (CRA) and carbapenem-resistant *Pseudomonas aeruginosa* (CRP) positive patients. For the qualitative section, healthcare providers of reported patients were interviewed about information exchange during patient transfers, precautionary measures and knowledge and beliefs concerning CPB.

Results

In total, 50 CPE-positive, 10 CRA-positive and 4 CRP-positive patients were reported during the inclusion period. Eighteen index-specific and 2 general interviews were conducted with 20 different care providers of 9 patients. The interviews revealed that, in most cases, information concerning the patient was transferred timely, but often a standardized method for sharing the information within and between institutions was lacking. Factors that enhanced care providers' motivation to adhere to precautionary measures were taking responsibility for the health of other patients, (pregnant) colleagues and for ones own health. Factors that reduced motivation were not acknowledging the relevance of the precautionary measures, a perceived negative impact of the measures on patients' recovery, differences in precautionary measures between healthcare settings and incomprehension for changes in precautionary measures.

Conclusions

CPB-positivity occurred more frequently than expected in the Dutch provinces of NH and FL. Standardizing the transference of information concerning CPB-positive patients, implementing transmural agreements, training personnel on CPB knowledge and

procedures, launching a national website on CPB and assigning one or several designated employees for CPB within healthcare institutions could improve communication between healthcare providers and thereby decrease the risk of CPB transmission.

BACKGROUND

Antimicrobial resistance (AMR) is a growing worldwide problem and an increasing public health threat(1). The World Health Organization (WHO) warns that new resistance mechanisms are emerging and that our ability to treat common infections is being threatened(2). It is estimated that in 2015, approximately 33,110 deaths attributable to infections with antibiotic-resistant bacteria occurred in the European Union(3).

The Netherlands belongs to the countries in Europe with the least infections caused by antibiotic resistant bacteria(3, 4). It is estimated that in 2015, approximately 5,000 infections with antibiotic-resistant bacteria occurred in the Netherlands, which resulted in 206 deaths attributable to AMR(3). Although there is still room for improvement, the current AMR situation in the Netherlands is encouraging. Firstly, physicians in the Netherlands are generally reserved in prescribing antibiotics(5). Secondly, the use of antibiotics in livestock has decreased with approximately 64% between 2009 and 2016(6). In addition, Dutch hospitals actively combat AMR through the Dutch search and destroy policy for methicillin-resistant *Staphylococcus aureus* (MRSA)(7) and the so called 'A-teams' (antimicrobial stewardship teams) for improving antibiotic stewardship of specialist physicians in hospital settings. Finally, there are multiple professional guidelines for preventing spread of AMR for both inpatient and outpatient settings(8). Nonetheless, the infection pressure of resistant bacteria from other countries(9), the environment, the food chain and within healthcare settings remains, occasionally resulting in outbreaks of resistant bacteria(10-12). From April 2012 to May 2018, a total of 212 outbreaks of resistant bacteria that were a threat to the continuity of care have been reported to the national early warning and response meeting of hospital-acquired infections and antimicrobial resistance (SO-ZI/AMR) in the Netherlands. Of these outbreaks, 44 were reported in the provinces of NH and FL(13). These outbreaks highlight the need for insight into risk situations for the spread of AMR to prevent increasing morbidity and mortality due to (further spread of) AMR.

Carbapenemase-producing *Enterobacteriaceae* (CPE), carbapenem-resistant *Acinetobacter spp*. (CRA) and carbapenem-resistant *Pseudomonas aeruginosa* (CRP) belong to the group of multidrug-resistant (MDR) bacteria and are categorized as priority-1 (CRITICAL) bacteria by the WHO(14). In healthcare, these carbapenemase-producing gram-negative bacteria (CPB) (as their susceptible analogous) most commonly are directly transmitted from patient to other patients through contaminated hands or through contaminated hands of healthcare workers due to physical contact with the patient. In addition, transmission can occur indirectly, through shared equipment or contaminated environmental surfaces(15). CPB are prevalent worldwide, however dif-

ferences in prevalence exist between and within continents and countries(16-20). In the Netherlands, patients are occasionally diagnosed with CBP, mainly following hospital admission abroad(21). However, based on data submitted by 28 Dutch laboratories to the Infectious diseases Surveillance System-Antibiotic Resistance (ISIS-AR), the overall prevalence of gradient test confirmed CPE has slightly increased (from 0.02% in 2014 to 0.05% in 2018 for *Escherichia coli* and from 0.25% to 0.52% in *Klebsiella pneumoniae*) (22). Even though CPE are still relatively rare in the Netherlands, they pose the biggest threat to public health, since infections with these bacteria leave very few therapeutic options(23, 24)and are often associated with prolonged hospitalization and increased mortality(25). Therefore, in April 2018, the Dutch minister of Health, Welfare and Sports decided that CPE should become mandatory notifiable as a category C item (to be reported on within one working day following diagnosis by the head of the laboratory to the public health service (PHS) in the Netherlands)(26, 27). This has taken effect as of July 1st 2019.

Various (medical) risk factors exist for CPB acquisition among hospitalized patients, the most important ones being the use of medical devices and carbapenem use(28). However, a situation in which the risk for the spread of CPB is also heightened, is during the transfer of a CPB-positive patient between different healthcare institutions or healthcare providers. When information on CPB-positive patients is inadequate or not shared timely, precautionary measures to prevent the spread of these bacteria are hampered. For healthcare providers, both in the inpatient 'cure' settings as in the outpatient 'care' settings, actual knowledge of precautionary measures and existing guidelines for CPB containment is increasingly important. Especially in the context of elderly living longer at home independently and being discharged sooner from hospitals nowadays. Due to these developments, the number of CPB-positive patients requiring care in outpatient settings is increasing. It is assumed that a knowledge gap concerning CPB exists in outpatient settings and that therefore an increase in knowledge and awareness concerning CPB is required of healthcare providers in these settings.

Therefore, we firstly aimed to gain insight into the frequency of CPB-positive patients in the Dutch provinces of Noord-Holland (NH) and Flevoland (FL) between February 2018 and February 2019 by means of a quantitative study component. Secondly, by means of a qualitative study component, we aimed to obtain a deeper understanding of the communication process between healthcare providers during transfers of CPB-positive patients and explore possible communication-related risk situations for the spread of CPB. The results of this study provide insight in the magnitude of diagnosed CPB-positive patients and the communication between healthcare providers involved. Therefore, the

study could contribute to the formulation of transmural agreements between institutions and healthcare providers regarding AMR and patient transfers in the Netherlands.

METHODS

Recruitment and sample

The Dutch provinces of NH and FL are divided into 6 PHS regions, in which a total of 14 laboratories provide diagnostics. All were approached to participate and agreed to voluntarily report CPE-positive patients anonymously to their regional PHS. Contact persons of the 6 PHS subsequently reported the cases to the research team of the study. Additionally, two laboratories voluntarily reported CRA/CRP-positive patients. During the recruitment period from February 2018 to February 2019, a total of 64 patients were reported to the research team. For each reported patient, one of the research nurses contacted the reporting medical microbiologist of the laboratory to obtain the name of the responsible healthcare provider of the patient (the one requesting the diagnostics from the laboratory and being the formal responsible for treatment and care). The nurse then contacted this responsible healthcare provider and requested them to obtain the patient's informed consent for following the patient's route in the healthcare system. After written informed consent was obtained from the patient, the research nurse planned an interview with the previously contacted primary responsible healthcare provider of the patient. Additionally, based on information obtained from the initial healthcare provider, other healthcare providers involved in the care of the CBP-positive patient were contacted by the research nurse and asked to participate (up to 5 healthcare providers per patient). Nurses asked when the patient was expected to leave the healthcare setting (e.g. transferred to another healthcare organization, or discharged), to be able to contact potential successive healthcare providers. To limit healthcare providers' time investment, verbal oral consent for participation was obtained at the start of the telephone interview.

Inclusion criteria

CBP-positive patients that were 18 years or older and received care (either in an institution or at home) were eligible for participation in the qualitative data collection. Healthcare providers were excluded when they were not able to remember the patient or when the patient was reported to the regional PHS ≥2 months after the CBP diagnosis.

Quantitative section

Laboratory detection

All participating laboratories perform CBP diagnostics according to the guideline of the Dutch Society for Medical Microbiology (in Dutch: Nederlandse Vereniging voor de Medische Microbiologie (NVMM)(29) and are ISO 15189 accredited(30). This prescribes the detection of carbapenemase production as a two-step procedure, of which the first step (screening) is performed by the diagnosing laboratories and the second step (phenotypic and genotypic confirmation) is mostly performed by the Dutch national institute for public health and the environment (RIVM). In short, screening occurs with a selective plate for CBP. When suspicious isolates prove to have a mean inhibitory concentration (MIC) >0,25 mg/L for meropenem, the elevated carbapenem MIC is confirmed with antibiotic gradient on a strip method. In most participating laboratories, when the strip method confirms a MIC >0.25mg/L, a PCR on known carbapenemase genes is performed and the strain is sent to the RIVM for phenotypic and genotypic confirmation. Some laboratories perform their own phenotypic confirmation by means of a CIM (carbapenem inactivation method) test(31).

Quantitative analyses

CBP cases were reported to the local PHS by email. Notifications included the name, sex and date of birth of the patients as well as the detected micro-organism. The local PHS forwarded the notification to the research team and KJ and GR processed the information in an excel sheet with all notifications. An index number was assigned to each patient and the qualitative data were obtained and processed by making use of the same index number. Descriptive analyses were performed to obtain the patients' age at time of reporting and the distribution of the different types of CBP that were detected. All analyses were conducted with Stata 13 (StataCorp., College Station, Texas, USA).

Qualitative section

Interview procedures

Semi-structured interviews were chosen as method of investigation, as they allow flexibility to explore new themes and can generate richer thematic data(32). All interviews were conducted in Dutch and were performed and conducted by telephone to minimize time investment of the healthcare providers. Two research nurses (KJ and GR) with previous qualitative interview experience conducted the interviews. At the beginning of the interview, the study was explained, patient and healthcare provider data were verified and oral informed consent of the healthcare providers was obtained. The following subjects were addressed during the interviews: information exchange during patient

transfers (what information was shared, in what manner, when, by who); precautionary measures (what measures were taken, on what grounds, were there barriers in applying these measures); the availability of guidelines; the healthcare provider's knowledge concerning CBP and need for more information concerning CBP; other healthcare providers that were involved in patient care (to plan additional interviews). An additional file shows the interview guide that was used (Additional file 1). The main goal of the interviews was to gain insight in the handlings of CBP in NH and FL and to reveal the information exchange between healthcare providers of CBP-positive patients, including barriers and enablers of patient related communication.

Qualitative analyses

To limit researchers' time investments, all interview recordings were transcribed by a processing agency. Consequently, the researchers entered the transcripts into a database using qualitative data analysis software (MAXQDA 2018). A descriptive content analysis was performed by two researchers (WV and ED) in an inductive manner. Subsequently, the two researchers discussed the content of the interviews and reached consensus on interpretation and important findings.

Ethical framework

This study was approved by the Medical Ethical Committee of the Academic Medical Centre of Amsterdam, the Netherlands (W17_384). In order to maximize confidentiality, all possible personal identifiers were removed from interview transcripts. Interview transcripts were only accessible to researchers from the research team. All respondents were able to withdraw consent to participate in the study at any time without clarification.

RESULTS

Quantitative section

Reported CBP-positive patients

In total, 50 CPE-positive patients were reported between February 2018 and February 2019. Additionally, two laboratories reported 10 CRA-positive patients and 4 CRP-positive patients. This resulted in a total of 64 patients, approximately two cases per 100,000 inhabitants of NH and LF. Fifty-nine patients (92%) were reported by seven laboratories and five patients were reported by another Dutch research group working on a CPE study. Of the reported patients, the majority was male (58%) and the median age at time of reporting was 69 (IQR 54-76). During the inclusion period of this study,

an outbreak of the CPE *Citrobacter freundii* occurred, which resulted in 22 *Citrobacter freundii* positive patients from 1 laboratory being included in our study. Therefore, most patients included in the study were diagnosed with *Citrobacter freundii* (36%), followed by *Escherichia coli* (17%) and *Klebsiella pneumoniae* (16%). Excluding the 22 outbreak patients, patients were most often diagnosed with *E. coli* (11/42, 26%), *K. pneumoniae* (10/42, 24%) and *Acinetobacter spp.* (10/42, 24%).

Of the 64 cases, 35 patients (55%) were not eligible for inclusion: 11 patients (17%) were reported at the PHS ≥2 months after diagnosis, nine patients (14%) did not receive care during their diagnosis, eight patients (13%) were deceased, five patients (8%) lived outside of the NH-FL area and two patients (3%) were <18 years of age. Of the 29 eligible patients (62% male, median age at time of reporting 67, IQR 54-75), in 12 patients (41%), care providers could not be reached within the study timeframe despite at least five attempts at different days and time periods. Furthermore, one patient (3%) was excluded because the care provider could not remember the patient, care providers of another 2 (7%) patients refused to approach the patient for participation and five patients (17%) did not consent to participation. Finally, eight CPE-positive patients and one CRA-positive patient consented to approach their healthcare providers. Of these nine patients included in the qualitative study, six were male (67%) and median age at time of reporting was 75 (IQR 59-78 years). Figure 1 shows the flowchart of the inclusion and exclusion of the patients.

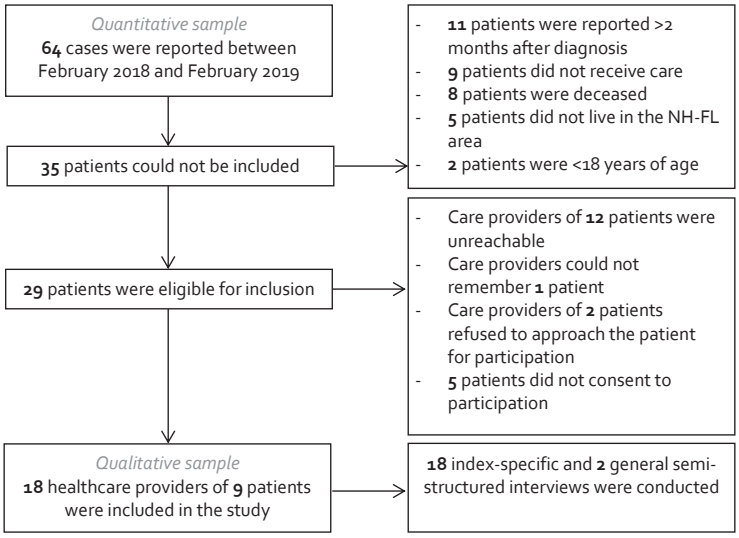

Figure 1. Flowchart of inclusion and exclusion of CPE/CRA/CRP-positive patients and their healthcare providers.
Abbreviations: NH = Noord-Holland; FL = Flevoland.

Qualitative section

Sample characteristics

In total, 18 index-specific and 2 general interviews (mean duration 20 minutes, ranging from 10 to 35 minutes) were conducted with 20 different care providers (70% female). For the index-specific interviews, the time from the patient being reported to the first interview ranged from 37 to 100 days. Eight of the 20 interviewees (40%) were specialist doctors (6 were hospital-based, 2 were general practitioners and 1 was based in a revalidation center). Eight interviewees (40%) were registered nurses (1 was hospital-based, 3 were employed at a revalidation center, 3 were nurses in home-based care and 1 was employed at a nursing home) (Table 1).

Table 1. Qualitative sample characteristics

Respondent number	Type of institution	Profession	Sex	Index number
1.	Revalidation center	Nurse	Female	1
2.	Home based care	Nurse	Female	2
3.	Revalidation center	Nurse	Female	3
4.	Revalidation center	Physical therapist	Female	
5.	Revalidation center	Occupational therapist	Male	
6.	Hospital	Physician	Male	4
7.	Revalidation center	Physician	Male	
8.	Home based care	Nurse	Female	
9.	Hospital	Medical fellow	Female	5
10.	Nursing home	Nurse	Female	
11.	Hospital	Nurse	Female	
12.	Hospital	Physician	Male	6
13.	Hospital	Physician	Female	
14.	Revalidation center	Healthcare assistant	Male	7
15.	Home based care	Nurse	Female	
16.	General practice	General practitioner	Female	
17.	Revalidation center	Nurse	Female	8
18.	General practice	General practitioner	Female	9
19.	Hospital	Infection prevention specialist	Female	None
20.	Hospital	Surgeon	Male	None

Figure 1. Flowchart of inclusion and exclusion of CPE/CRA/CRP-positive patients and their healthcare providers. Abbreviations: NH = Noord-Holland; FL = Flevoland.

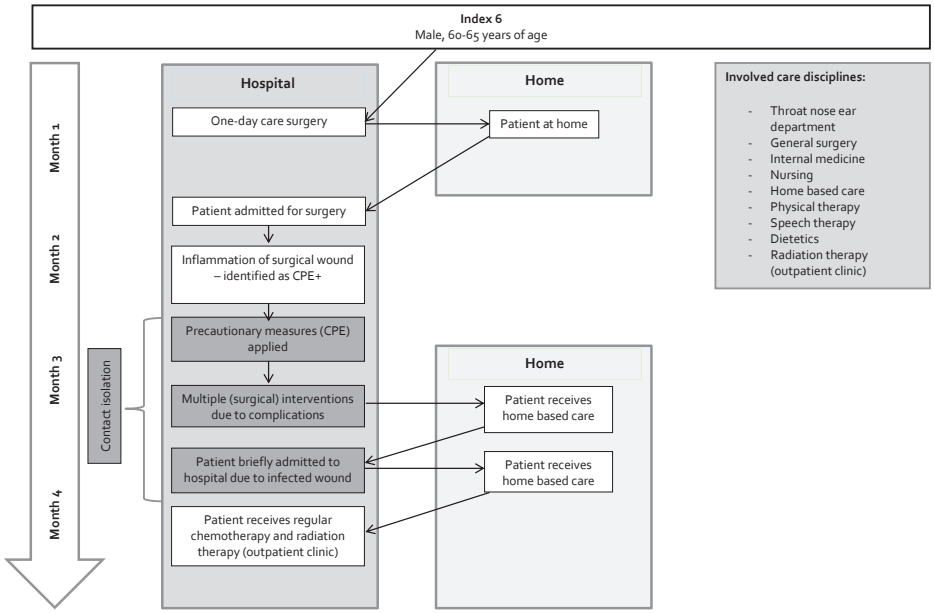

Figure 2. Example of cascade of care and involved care disciplines.
Abbreviations: CPE – Carbapenem-producing *Enterobacteriaceae.*

Complex care networks

The personal cascades of care varied considerably between patients. For all 9 included patients, multiple care providers within one institution were involved in the care of the patient. In 70% of the included cases, multiple care providers within one institution and from different institutions were involved. Furthermore, patients were often transferred from one institution to another or discharged from an institution within a short period of time. Figure 2 provides an example of the complex care network and the abundance of involved care disciplines of one of the included patients, demonstrating the theoretical potential of spread of CPB within and between healthcare settings and care providers.

Interview findings

Communication

The interviews revealed that, in most cases, information concerning the CPE/CRA-positive patient was shared timely (e.g. before the patient was transferred or immediately after the patient was found to be CPE/CRA-positive). Three care providers indicated that information was not accessible in time which resulted in a state of confusion.

"Yes, I can remember that it [the sharing of information] was at a later time point, that I had already been there [the patient] once [after the patient was diagnosed with CPE] and that I did not apply contact isolation that first time." (respondent number 1, general practitioner)

Methods for the transference of information were several and differed between and within institutions. Communication methods entailed transfer letters, telephone, e-mail, face-to-face conversations, work meetings and electronic transfer dossier (POINT). For some patients, multiple methods of information transference were used (information was shared face-to-face and through email, information letter and personal documents of patient). In almost all cases, the CPE/CRA-positivity of patients was made known by signs on the door of the patients' room and by a pop-up in the electronic patient dossier (EPD). As mentioned before, most of the healthcare providers received the information in time and were satisfied with the information. This was especially true for healthcare providers in institutional care, since these institutions often had a designated employee responsible for infection prevention. In these cases, the infection prevention measures in case of a CPE/CRA-positive patient were known and easily applied according to the interviewees.

The interviews also uncovered several suboptimal aspects of the communication about CPE/CRA-positive patients. In almost all interviews, the transference of information proved not to be clearly arranged, since no standardized method and route for sharing the information within and between institutions exist. This resulted in confusion among care providers and most of them were not able to reproduce how and when the information concerning the CPE/CRA-positive patient was transferred to them. Furthermore, sharing information concerning CPE/CRA-positivity and aligning infection prevention measures did not routinely include disciplines or departments such as cleaning and food distribution. For many care providers, this was the first time they encountered a CPE/CRA-positive patient in their career. Therefore, in many cases, care providers felt the need to look up extra information on CPE/CRA and infection prevention measures. In some interviews, a passive attitude towards these infection prevention measures was observed: care providers did only acknowledge that precautions had to be taken when seeing a sign on the door of a patient or when gloves were placed in the room of a patient.

"The patient told me that the home-based care nurses wore aprons with gloves, and they [aprons and gloves] were present there [the patients' house] so then I used those during the second visit." (respondent number 16, general practitioner)

In extramural care, for instance in home-based care, a specialized infection prevention employee was often lacking. So, home-based care nurses had to find out the necessary infection prevention measures themselves. Multiple care providers indicated that they would trust information shared by a specialized employee from the hospital the most. However, the hospital is not always involved in diagnostics and care for these patients. In some interviews, it appeared that care providers were completely dependent on the information that was shared with them by specialized employees, whereas the sender of that information was not always aware of his/her position and relevance in sharing information.

"By now I know some [resistant bacteria] by heart, but I don't know this one [CPE] top of mind. So I completely rely on the advise of the medical microbiologist and the infection prevention mostly." (respondent number 13, physician hospital)

Motivation for applying infection prevention measures

The interviews showed that the level of motivation of care providers to adhere to infection prevention measures differed. Factors that increased care providers motivation to apply infection prevention measures were taking responsibility for their own health, for other patients or for (pregnant) colleagues. However, factors that undermined care providers motivation to apply infection prevention measures were also reported. Some care providers did not see the relevance of the measures and multiple care providers indicated that the infection prevention measures negatively impacted the treatment of patients since they were restricted in moving around through the institution or in exercising (for instance in revalidation care). This subsequently lead to irritation and lack of understanding of measures by patients.

"It [infection prevention measures] often results in anger, irritation. We see revalidation programs that turn out different than normally. Patients can't always go to the physical therapist when they want to, or exercise themselves or do this or do that." (respondent number 7, physician revalidation center)

Furthermore, multiple respondents indicated that differences in infection prevention measures between settings (admitted to hospital vs. outpatient hospital visit), between care providers and between care providers and family members caused confusion and lead to incomprehension for the advised measures.

"We [care providers of the patient] were surprised that, according to the infection prevention specialists, this [outpatient hospital visit] was allowed without isolation precautions." (respondent number 12, physician hospital)

Critique among healthcare providers also arose when different measures were advised for the period a MDR bacterium was only suspected (for instance when a patient had recently been hospitalized abroad) and following the CPE/CRA-positive diagnosis. This could for example result in the shift from contact isolation to strict isolation when CPE/CRA-positivity was confirmed. Care providers did not always seem to realize that part of the infection prevention measures were instituted to protect other patients. In a few other cases, care providers expressed concern because they believed that the intensity of infection prevention measures was too low and they were skeptical about the effectiveness of the measures.

"I believe that, despite everything we know about why she [the patient] is in isolation, we should wear protective clothing at all times … because unconsciously you carry it [CPE] with you … I think that both nurses and visitors should adhere to the same rules, because unconsciously it [CPE] is present between the sheets and it [CPE] appears everywhere." (respondent number 1, nurse revalidation center)

Knowledge and beliefs

The interviews revealed a contradiction in the perceived severity of CPE/CRA-positivity and divergent beliefs on CPE/CRA. Many care providers seemed to take CPE/CRA-positivity seriously and were motivated to enhance their knowledge about CPE/CRA prevention. However, their confidence in measures was limited: multiple respondents indicated that pregnant or vulnerable colleagues, and colleagues with small children were deliberately not involved in the care for CPE/CRA-positive patients.

"But also fear for if I also have this bacterium and I am unaware of that, how will that go at home? What do I get from that? You know, I rather not have that, because I have a small child at home and I have family members that are pregnant, you know?" (respondent number 17, nurse revalidation center)

"Colleagues that feel sick or are vulnerable in terms of health, or we have a colleague that is pregnant, they are definitely not employed [in the care for CPE-positive patients]." (respondent number 2, nurse home- based care)

Additionally, multiple respondents indicated the need for CPE/CRA-specific guidelines, including specific advice for the aforementioned pregnant or vulnerable colleagues, and colleagues with small children.

"There are no specific guidelines for us [general practitioners], so I think that is a real issue. Mainly for practical reasons: how do you deal with that [CPE positive patients], how

long does it [CPE positivity] take, is there a limit for the contact isolation or should it be continued completely?" (respondent number 16, general practitioner)

Often, there were initiatives of CPE-related training or education when CPE/CRA-positive patients were admitted to the institution. Some care providers indicated that the extra attention for and information about CPE/CRA by means of education was appreciated, took away concern, and that the training matched the expectations and needs.

"We had clinical training by the hygienists about that [CPE] … it was very clear why certain measures had to be taken … A clinical training takes away stress, concern and questions … They explained it to us in understandable language, for all levels of personnel that are employed at our institution. So for individuals working in the kitchen, but also for all nurses." (respondent number 17, nurse revalidation center)

In some cases, the follow-up policy for a CPE/CRA-positive patient was clear and in these cases care providers shared extensive information about the patient, the procedures and infection prevention advices with subsequential care providers.

"Yes, I wrote a letter about what happened during the admission and what the result of the cultures was and how we deal with that and what the advices are to implement there [at subsequent institution]." (respondent number 9, medical fellow hospital)

In one case, infection prevention materials were given to the patient for the home-based care employees to enable infection prevention measures.

"*We always provide the patient with a few aprons for the home-based care employees." (respondent number 14, healthcare assistant revalidation center)*

Several other inadequacies in the CPE/CRA knowledge of care providers were also observed. Some care providers thought that CPE/CRA was a virus, others thought that CPE/CRA is abundantly present on and around a CPE/CRA-positive patient (for instance on skin and other body parts, in clothes, between sheets or in the entire room) and that transmission can occur in numerous ways (for instance when hugging family, shaking hands or standing in an elevator).

"*When such a man [CPE positive patient] comes outside, and he carries such a dangerous organism and he is standing in an elevator … and he goes home and hugs his wife, to what extent is that dangerous? And I don't know much about that in specifically this bacteria [CPE]." (respondent number 12, physician hospital)*

Many care providers were unaware of the follow-up policy for CPE/CRA-positive patients in terms of both follow-up testing and sharing of information with simultaneously involved or future care providers when a patient was discharged. Often the CPE/CRA status of the patient was communicated when transferring the patient to a subsequent care provider, but advice on how to act was not.

"I do think there is a clear policy in the hospital. But what happens when a patient is discharged… Yes, then you are curious: what about all that?" (respondent number 12, physician hospital)

"Well, he [the patient] and his wife were able to tell what was wrong with him very well themselves. Yes, they talked a lot about the bacterium that he carried, so I am not sure whether my colleagues called the hospital where he was also a patient upon discharge, but if we did not tell them, I can guarantee you that they [the patient and his wife] would announce it themselves." (respondent number 17, nurse revalidation center)

Almost all respondents indicated that guidelines or protocols for MDR bacteria exist within the organization. However, specific guidelines and protocols for CPE/CRA-positivity are often lacking. Even when guidelines and protocols are present within an organization, care providers are frequently not certain where these guidelines can be found. In institutional care, care providers often indicated to contact infection prevention specialists when their knowledge concerning CPE/CRA-positivity and infection prevention measures was insufficient. Almost all respondents indicated the need for extra information and education concerning (the care for) CPE/CRA-positive patients. Some care providers preferred information solely about the bacteria and mechanisms of resistance, and others preferred information merely about the required infection prevention precautions (why do measures need to be taken vs. what measures need to be taken).

DISCUSSION

The purpose of this study was to firstly gain insight into the frequency of CPB-positive patients in the Dutch provinces of NH and FL by means of a quantitative component. Secondly, by means of a qualitative component, we aimed to obtain a deeper understanding of the communication process between healthcare providers during transfers of CPB-positive patients and explore possible communication-related risk situations for the spread of CPB. For the quantitative section of this study. a total of 64 patients were reported in NH and FL from February 2018 to February 2019. Our study shows that in

case of the 18 healthcare providers included in the qualitative section of the study, the information regarding a CPE/CRA-positive patient being transferred was mostly shared timely. However, methods for the transference of information were diverse and in almost all cases, the transference of information was not standardized. Many care providers could not exactly recall how and by who the information was shared with them. The motivation to adhere to precautionary measures in case of a CPE/CRA-positive patient differed between care providers. Factors that enhance motivation were taking responsibility for the health of other patients, for (pregnant) colleagues and for ones own health. Factors that reduce motivation were not acknowledging the relevance of the precautionary measures, a perceived negative impact of the precautionary measures on the patients' recovery, differences in precautionary measures between healthcare settings and incomprehension for the possible shift in previous advised precautionary measures. Most care providers had not encountered CPE/CRA-positive patients before and in the majority of cases, the follow-up policy for the CPE/CRA-positive patient was unclear. Almost all care providers indicated that they took CPE/CRA-positivity seriously and specified that they felt the need to obtain more information concerning CPE/CRA-positivity.

Since CPE are still relatively rare in the Netherlands and other European countries(3, 17, 19, 20, 22), based on estimates of medical microbiologists we expected to find 15 to 20 CPE-positive patients in NH and FL in one year. Even when not taking the 22 related outbreak patients into account, the total of 28 other CPE-positive patients that were reported was substantially higher than expected. As the prevalence of CPE is rising worldwide(3, 33-35), our findings might suggest that the prevalence is also rising in the Netherlands. Indeed, national surveillance data from ISIS-AR show that confirmed non-susceptibility in *E. coli* and *K. pneumoniae* isolates was low but slightly increasing over the past 5 years (0.05% and 0.52% in 2018, respectively)(22).

In line with our expectations, we found that experience with CPE/CRA-positivity and applicability of precautionary measures was more present in the 'cure' settings, since AMR has been a subject of interest in these settings for a longer time and consequences of AMR for the vulnerable (inpatient) population can be severe. However, in public health, or 'care' settings, we found that healthcare providers have not been confronted with MDR bacteria regularly, so experience with CPE/CRA and applicability of precautionary measures was found to be lower. Our study showed that for most care providers, this was the first time they encountered a CPE/CRA-positive patient and that the transference of information regarding a CPE/CRA-positive patient was not clearly arranged.

Also, adequate knowledge on CPE/CRA was often lacking. Multiple care providers indicated the need for more information on CPE/CRA. Often, a single moment of extra education for healthcare providers was organized within an institution when a CPE/CRA-positive patient was encountered. Even though the training met expectations and needs of care providers, the single moment of education could lead to a delay in application of adequate measures by the care providers from the moment a CPE/CRA-positive patient was diagnosed. These findings advocate the need for structurally training medical staff on CPE/CRA. To maximize understanding and optimize compliance with infection prevention measures, the training should emphasize the substantiated and consciously formulated differences between guidelines for different healthcare settings. Training on CPE/CRA could actively involve care providers, for instance through e-learnings on CPE/CRA. E-learnings are accessible at all times and can reduce delay in education when a CPE/CRA-positive patient is admitted or encountered. Also, to guarantee sufficient knowledge is present within an institution at all times, we suggest to appoint one or several designated individuals within healthcare institutions that possess knowledge on CPE/CRA and who actively inform others within the institution in case of CPE/CRA-positive patients. Our findings on the importance of adequate knowledge and perceived severity among healthcare providers correlate with findings from a study exploring barriers and enablers to MRSA admission screening in hospitals (36) and a study into the acceptability of screening for CPE(37).

Furthermore, it is advised to standardize patient transfer information (for instance through electronic transfer dossier) in which it is obliged to indicate whether or not a patient carries a resistant bacterium. Finally, these findings indicate the need for uniform, transmural agreements concerning CPE/CRA-positive patients. Constituting transmural agreements has been described as one of the tasks for the 10 regional antimicrobial resistance networks in the Netherlands. These networks were commissioned by the Dutch Ministry of Health, Welfare and Sport in 2016 and have been formally implemented in May 2017.

Various care providers specified the need for the development of a CPE/CRA-specific guideline by the Dutch General Practitioner Society (in Dutch: Nederlandse Huisartsen Genootschap (NHG)) to help general practitioners in optimizing care for CPE/CRA-positive patients. However, since NHG guidelines are specifically for general practitioners, we additionally advocate for the expansion of the multidisciplinary guideline on highly resistant microorganisms by the National Coordination of Infectious Disease Control (in Dutch: Landelijke Coördinatie Infectieziektebestrijding (LCI)) which is applicable for all care providers. This guideline should include information and hands-on advise for care providers on the diagnosis, treatment and care for CPE/CRA-positive patients and

their families. The guideline should emphasize why differences in infection prevention measures exist between settings and bacteria since incomprehension might lead to non compliance.

Moreover, we believe that an integrated national website on CPE/CRA should be developed. This website should include clear patient information folders and a realistic risk assessment about the severity of CPE/CRA-positivity and infections for both patients, household contacts/visitors, and care providers. The information on the website should be comprehensible for patients of all educational levels. This recommendation correlates with findings from a recent study into patient experiences concerning hospital screening for CPE, which highlighted the need for access to clear patient information on CPE(38).

We believe that the major strength of our study is the mixed-methods design. This has allowed us to both provide insight into the number of CPB-positive patients within the provinces of NH and FL and explore themes within communication of healthcare providers that could potentially influence the transmission of CPB within and between healthcare institutions. However, our study also had several limitations. Firstly, the diagnosing laboratories of NH and FL voluntarily reported CPE-positive patients. Only one participating laboratory automated the reporting of CPE-positive patients, all other laboratories manually reported CPE-positive patients to the research team. This might have resulted in not all diagnosed patients being reported to the research team and an underestimation of the true number of CPE-positive patients in NH and FL. However, the number of patients reported to the research team did not substantially deviate from the number of patients reported from the same laboratories to the National Institute for the Public Health and the Environment (RIVM) for surveillance for those regions. Nonetheless, reporting of CPE-positive patients to the RIVM is also on a voluntary basis and not all laboratories that provide diagnostics for the NH and FL region participate in this surveillance. It is therefore likely that underreporting and thereby an underestimation of the true number of CPE-positive patients in NH and FL has actually occurred. An underestimation of the true number of CRA and CRP patients has most likely occurred, since only two laboratories reported these bacteria and they are included in the study as an additional finding. Also, we were unable to include 20 out of 29 patients that were eligible for inclusion in the qualitative section. The 20 patients that were eligible but were not included were more often female and were younger compared to the eligible patients that were included, which could have lead to participation bias. However, we do not believe that the care for the eligible, not included patients differs from the care for the eligible, included patients and therefore the effect of the participation bias on the study results is expected to be negligible. Furthermore, partly due to the limited

inclusion period, we were only able to include healthcare providers of 9 patients in the qualitative section of the study and we were unable to include multiple healthcare providers for 4 patients. However, we do believe that the 20 interviews concerning the 9 included patients provided us with valuable insights into communication of care providers concerning CPE/CRA-positive patients. It is expected that healthcare providers will be more inclined to participate in comparable studies since CPE-positivity became mandatory notifiable on July 1st 2019. Also, the time interval for reporting CPE-positive patients will decrease from July 1st 2019 because the accepted period of reporting has been legally maximized at one working day. Moreover, mainly due to the mixed-methods design of the study and the limited inclusion period, a relatively limited content analysis of the qualitative data was performed resulting in limited conceptual interpretation. However, we do believe that valuable information was derived from the interviews with the healthcare providers. Future strongly designed qualitative studies could be performed to confirm and validate our findings. Due to the semi-structured character of the interviews, we cannot exclude interperson variability in the interviews. However, we tried to minimize variability by simultaneously providing both research nurses with interview instructions, during which questions were addressed and uncertainties were resolved. Furthermore, both nurses made use of the same interview guide.

Conclusions

CPE-positivity occurred more frequently than expected in the Dutch provinces of NH and FL. Most care providers are not used to caring for CPE/CRA-positive patients and adequate knowledge concerning CPE/CRA is often lacking. Standardizing the transference of information concerning CPE/CRA-positive patients could improve communication between healthcare providers and thereby decrease the risk of CPE/CRA transmission during patient transfers. This would ideally include at least automated timely data exchange (among others of the CPE/CRA status and easily accessible information on necessary precautionary measures) between institutions before transfer actually takes place. Furthermore, formal and mutually consented transmural agreements could contribute to optimizing communication about CPE/CRA-positive patients. Additionally, care providers' knowledge about CPE/CRA and advised precautionary measures could be enhanced by means of E-learnings, and a national website on CPE/CRA could offer information to both care providers and CPE/CRA-positive patients. Finally, one or several designated employees for CPB or MDR bacteria in general within an institution should be responsible for maintaining and sharing knowledge (also during MDR cases or outbreaks) within the institution. In our opinion, the findings of our study are transferable to other regions of the Netherlands. Transferability to other countries is dependent on the healthcare infrastructure (for instance whether cure and care domains are separated as is the case in the Netherlands). Future qualitative studies could deepen

our understanding of the importance of communication between healthcare providers in battling AMR worldwide. Furthermore, future studies should focus on the feasibility of the implementation of proposed recommendations (drivers and barriers) in various healthcare settings and institutions.

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Conflicts of interest

The authors declare that they have no competing interests.

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SUPPLEMENTARY MATERIAL

Interview guide

Research question:

What are potential risk situations for the spread of CPB in communication between healthcare providers during transfers of CPB-positive patients?

Interview questions:

- Introduction:
  - Can you explain me your role in the care for this patient?
  - When were you first involved in the care for this patient?
- Information exchange during patient transfers:
  - Can you indicate how you have been informed about this patient (by whom, in what manner (written/by telephone/digital), when (before/after the transfer/the same day))
  - Was it immediately clear that you were dealing with a CPB-positive patient?
- Precautionary measures:
  - What precautionary measures have been taken for this patient?
  - Can you indicate the grounds on which these decisions have been made?
  - Have you encountered any barriers in applying these measures?
- Availability of guidelines:
  - Are there any precautionary measure guidelines within your institution that you have consulted for this patient?
  - If yes, are these guidelines easy to apply?
- Knowledge of healthcare provider:
  - Were you already familiar with CPB?
  - Would you need additional information and advice on CPB and necessary precautionary measures?
  - If yes, what kind of information/advice?
  - From whom and in what manner would you prefer to receive additional information and advice?
  - When would you prefer to receive additional information and advice?
- Other healthcare providers:
  - Are there any other healthcare providers involved in the care for this patient?

8

General discussion

The overall aim of this thesis was to describe the occurrence of and associated factors with antibiotic resistance among specific sociodemographic groups living in Amsterdam, the Netherlands. The results from these studies allow a more specific understanding of the public health needs for these groups. This thesis focused on (I) antibiotic knowledge, antibiotic use and prevalence of MRSA among different ethnic groups, (II) prevalence of ESBL-E among MSM and residents of long-term care facilities, and (III) perceived barriers and facilitating factors for preventing the spread of carbapenemase producing gram-negative bacteria during patient transfers between healthcare providers. Table 1 summarizes the main conclusions and recommendations of this thesis.

Table 1. Summary of principal findings and recommendations in this thesis

Chapter	Demographic group	Conclusions	Recommendations
2	Ethnic groups in Amsterdam	Lower antibiotic knowledge among all ethnic minority groups compared to the Dutch group, while some ethnic groups more frequently used antibiotics Level of antibiotic knowledge was not associated with antibiotic use	Study the reasons behind variation in antibiotic use among different ethnic groups using mixed methods studies Perform a living (e.g. yearly updated) systematic review on ABR with special attention for differences in subgroups of the community Identify how different ethnic groups can be reached and served for prevention Launch a differentiated prevention message to educate individuals from different ethnic groups on antibiotics and ABR Increase physicians' awareness of ethnic differences in antibiotic use
3	Ethnic groups in Amsterdam	Antibiotic knowledge could be measured by a 4-item questionnaire on appropriate antibiotic use in large-scale studies in which measuring antibiotic knowledge is part of a broader array of research questions	Studies aimed solely to measure antibiotic knowledge should use a more extensive, validated questionnaire on antibiotic knowledge
4	Undocumented migrants and uninsured legal residents	The prevalence of nasal MRSA carriage was 2.0% among undocumented migrants and uninsured legal residents, relatively higher compared to the general Dutch population, but lower than found in previous studies among migrants and asylum seekers	Investigate MRSA carriage among broader migrant populations (both legal and illegal and both residing in urban and rural areas) Periodical surveillance of MRSA carriage among all migrants, since distribution of migrants and migration patterns are likely to change in the future

Table 1. Summary of principal findings and recommendations in this thesis *(continued)*

Chapter	Demographic group	Conclusions	Recommendations
5	MSM	The prevalence of rectal ESBL-E carriage was 16.3% among MSM ESBL-E carriage was associated with a high number of partners and practicing certain sexual behaviors (especially rimming) with casual partners	Inform health care professionals about increased ESBL-E prevalence and possible sexual transmission among sexually active MSM Inform MSM about ESBL-E and possible sexual transmission through existing STI information sources (such as Soa Aids Nederland) and online dating apps using gamification Include information on increased prevalence of ESBL-E among MSM and its association with sexual activity in guidelines for empirical antibacterial therapy of sepsis in adults
6	Residents of LTCF	The prevalence of MDR microorganisms was 18.2% among residents of LTCF with large variation (0%-47%).	Identify structural differences in infection control practices between LTCF to direct interventions to LTCF with low infection control practices Periodical surveillance of colonization with resistant microorganisms among residents of LTCF through an additional module in SNIV Introduce less labor intensive surveillance methods such as sewage water measurements or LQAS in SNIV Transfer of data on LTCF from the Dutch Healthcare Inspectorate to SNIV
7	Healthcare providers of CPB-positive patients	Healthcare providers were not used to caring for CPB-positive patients because of their relatively rare occurrence Knowledge concerning CPB among healthcare providers was low	Standardize transfer of information concerning CPB-positive patients between healthcare institutions Enhance healthcare providers' knowledge on CPB Appoint dedicated employees for ABR management within healthcare institutions Make specific CPB transmural agreements

Abbreviations: ABR – antibiotic resistance; MRSA – methicillin-resistant *Staphylococcus aureus*; ESBL-E - extended spectrum β-lactamase producing *Enterobacterales*; MSM – men who have sex with men; LTCF - long-term care facilities; MDR – multidrug resistant; LQAS – lot quality assurance sampling; SNIV – surveillance network infectious diseases nursing homes; CPB - carbapenemase producing gram-negative bacteria

ANTIBIOTIC KNOWLEDGE AND ANTIBIOTIC USE AMONG DIFFERENT ETHNIC GROUPS

Studies from multiple countries worldwide suggest that differences in various aspects of antibiotic knowledge and antibiotic use according to ethnicity do exist. However, the studied aspects of knowledge and use, as well as the studied populations vary greatly between studies. Also, these studies did not compare knowledge among large ethnic groups with knowledge among the general population. A study from New Zealand indicated that antibiotic knowledge significantly differed among Indian, Egyptian and Korean individuals living in New Zealand, with Korean individuals having lower levels of knowledge compared to Indian and Egyptian individuals(1). An Australian study reported on knowledge, attitudes and perceptions regarding antibiotic use and self-medication among Australian Chinese migrants and showed that 70% of participants would stop using antibiotics when symptoms improved and 61% would use leftover antibiotics for similar symptoms(2). A nationwide-survey from the United States showed that white individuals reported twice as many antibiotic drug prescriptions per capita compared to individuals from other ethnicities(3). Another study from the United States disclosed Latino and Asian parents being 17% more likely to report that antibiotics were definitely or probably necessary for treating their children compared to non-Hispanic white parents(4). However, a later study among pediatric emergency departments in the United States showed that non-Hispanic black and Hispanic children were less likely to receive antibiotics for viral acute respiratory tract infections compared to non-Hispanic white children(5), in concordance with others (3).

In **Chapter 2**, we found that in Amsterdam, the Netherlands, antibiotic knowledge as measured by a 5-item questionnaire on appropriate antibiotic use was lower among all ethnic minority groups compared to Dutch, with second generation ethnic minorities showing higher levels of knowledge compared to first generation migrants. Our findings suggest that there are aspects prohibiting individuals from ethnic minority groups from learning about appropriate antibiotic use. Possibly, they may have never had the opportunity to learn about antibiotic use, experience language barriers, have lower health literacy, hold on to habits from their home countries or do not understand or were never told about when and how to apply antibiotics by their physician. These factors may also account for high inappropriate antibiotic use among ethnic groups in other studies, such as the aforementioned Australian study in which inappropriate antibiotic use was more common among Chinese migrants(2). Additionally, we found ethnic differences in the use of antibiotics, with a higher proportion having received at least one prescription, but a lower mean number of antibiotic prescriptions among some ethnic minority groups compared to the Dutch group. This latter finding corresponds to the

aforementioned study from the United States, which illustrated higher antibiotic use among white Americans compared to Americans from other ethnicities(3). Differences in antibiotic use can result from varying prescribing practices by physicians or ethnic minority groups may more often purchase antibiotics online or in their country of origin. Since in our study data on antibiotic use were obtained by linking study data to healthcare insurance data, antibiotics obtained outside of regular healthcare will be missed. Of interest, we showed that a lower level of antibiotic knowledge was not associated with receiving antibiotics or average number of antibiotic prescriptions, and ethnic differences in antibiotic use therefore could not be solely explained by level of antibiotic knowledge. One other Dutch study did however show higher use of antibiotics (data obtained from electronic patient files by general practitioners) among first generation migrants from Turkey, Morocco, Surinam or the Antilles compared to Dutch(6). To date, no other studies have evaluated antibiotic knowledge and antibiotic use according to ethnicity in the Netherlands. Given the size of the HELIUS study, we believe future studies should not focus on confirming our study, but mixed methods studies should focus on explaining why differences in antibiotic knowledge and antibiotic use among ethnic groups exist.

One way to improve antibiotic knowledge and reduce inappropriate use of antibiotics would be through prevention campaigns. Informing the general public on antibiotic resistance by means of an awareness campaign was part of the 2015-2019 antibiotic resistance program of the Ministry of Health, Welfare and Sports(7). The campaign was in Dutch and was repeated in 2015, 2016, 2017 and 2019 and included radio commercials, pre-rolls (movies that appear prior to for instance YouTube movies), banners, posters, folders, a special edition of a Belgian comic strip (‘Suske and Wiske’), attention for antibiotic resistance in multiple museums and during the national Antibiotic Awareness day. Focus of the campaign was to inform the public not to use antibiotics to treat the common cold, influenza or stomach flu, not to use leftover antibiotics, not to use antibiotics from family/friends, to finish the advised antibiotic course and to pay attention to personal hygiene.

The effect of the 2019 campaign was evaluated by means of a campaign effect study performed by a survey among a small subsample of the general adult population. It is unknown whether culturally diverse groups were reached by the campaign. The effect study showed that almost all respondents were aware that antibiotic resistance might affect themselves, with numbers comparable before and after the campaign. Also, a comparable number of participants before and after the campaign were aware that antibiotics should only be used for bacterial infections. Furthermore, a similar proportion of participants wrongfully assumed antibiotics could also be used for fungal or

viral infections before and after the campaign. The campaign thus was unsuccessful in increasing knowledge on antibiotics only being effective for bacterial infections. Also, the campaign was unable to further stimulate individuals to abide to action perspectives (only using antibiotics when indicated, taking the prescribed dosage at the correct time, finishing prescriptions, not using left-over antibiotics, washing hands before and after preparing food, washing hands after visiting the toilet and washing hands after being in contact with pets or other animals)(8). However, the campaign effect study was conducted in a small group of participants and only consisted of a simple questionnaire before and after broadcasting the radio commercials (the campaign effect study did not measure effects of the other components of the campaign). In our opinion, the study was therefore not scientifically sound enough to reliably determine the effect of the campaign.

Reasons for the campaign assumed not being unsuccessful were not identified, but recommendations for future campaigns were presented. Firstly, future campaigns should focus on certain action perspectives where further improvement is desirable, such as washing hands after for instance being in contact with pets and animals and before cooking and eating, not using leftover antibiotics, only stopping prescriptions after consulting a physician and taking the prescribed dosage at the correct time and not skipping dosages. Secondly, future campaigns should consider only including one action perspective per campaign visual or uttering. In our opinion, focusing on one component per campaign could indeed improve the understanding of the campaign.

Possibly, the described campaign was unsuccessful because it focused on the entirety of the Dutch population and was not specifically targeted to fit different groups. Reaching culturally diverse populations with effective health communication remains a widely acknowledged issue(9). However, based on our study described in **chapter 2** we do propose a differentiated prevention message tailored to inform individuals from different ethnic groups. Since none of the ethnic groups are culturally identical, the message should be customized to fit the needs of separate groups and should focus on specified misconceptions among individuals from different ethnic groups. For instance, as shown in **chapter 2**, the proportion of Ghanaian individuals that wrongfully believe antibiotics are effective in treating influenza (28%) is much higher compared to the proportion of Dutch individuals that share this belief (7%), whereas Dutch individuals more often unjustly believed antibiotics are effective in treating bronchitis (49%) compared to Turkish individuals (33%). Based on our experiences at the Public Health Service, we believe that interviews with community leaders, key figures, performed by interviewers that speak the same language, as well as with GPs and their practice assistants and nurse practitioners, can provide valuable insights into how the message should be trans-

ferred and what narrative should be used. Also, based on previous experiences, there should be constant room for dialogue between sender and receiver of the message. An elaborate social media approach targeting technologically adept individuals through e.g. Instagram, Facebook, YouTube, Snapchat, Twitter or TikTok should be used to ensure that targeted individuals of all ages, including both first and second generation migrants, can be reached. We believe that the prevention message should be designed in co-creation with the targeted groups. Additionally, infectious disease specialists from local Public Health Services, organizations specialized in reaching ethnic minority groups (for instance Pharos), the ten regional care networks for antibiotic resistance, the National Institute for Public Health and the Environment (RIVM) and the Ministry for Health, Welfare and Sport should collaborate to ensure a scientifically sound and practically useful prevention message. Finally, a rigorous, quantitative and qualitative evaluation of the effect of the prevention message should be performed.

We believe that the proposed prevention message should be preceded by a living systematic review (a review that is frequently, for instance yearly, updated) of the most recent scientific evidence on antimicrobial policies with special attention for differences in subgroups of the community. The data on antimicrobial resistance are constantly changing and new insights in emerging resistance mechanisms, optimal antibiotic dosages and correct use of antibiotics are frequently reported. It is important that those recent insights are incorporated in the prevention message.

Nonetheless, as we showed in **chapter 2**, level of antibiotic knowledge was not associated with receiving antibiotics or average number of antibiotic prescriptions. This might indicate that lower antibiotic knowledge among patients might not necessarily lead to increased antibiotic use after all. Possibly, other factors than patients' antibiotic knowledge, such as differences in social norms (for instance expecting a prescription when visiting the general practitioner), differences in incidence of bacterial infections or physicians' motivations to prescribe antibiotics could drive differences in antibiotic use among ethnic groups(1, 6, 10-12). Differences in the incidence of bacterial infections could occur in line with socioeconomic differences, rendering those with poor living conditions or living in poverty more vulnerable for bacterial infections and in increased need for antibiotics(13). Even though we did include several comorbidities in our study, data on bacterial infections was not available. Future scientific studies should focus on unravelling the reasons behind the varying antibiotic use among different ethnic groups.

Finally, physicians should be informed that ethnic differences in antibiotic use exist and should be made aware of their possible leniency or reluctancy to prescribe antibiotics to those who ask for it or to those from specific ethnic groups. If made aware of such

behaviors, physicians could focus more on guideline-based equal prescribing practices across all ethnicities. Also, physicians should be motivated to educate their patients why antibiotics may not be warranted for viral infections and help ensure that their patients know how to correctly use antibiotics. Because of their close collaboration with health-care providers, the ten Dutch regional care networks for antibiotic resistance could play an important role in this nationwide awareness message for physicians. The ABR care networks could for instance organize meetings for general practitioners in their regions to ensure this important message reaches general practitioners throughout the country.

In **Chapter 3**, we asked how the concept of antibiotic knowledge changes across ethnicities and to do so, we analyzed the psychometric properties of the items used to measure antibiotic knowledge in **chapter 2** and evaluated whether these questions are suitable to measure antibiotic knowledge across ethnicities. We initially used a 5-item questionnaire on antibiotic knowledge, but found two items (i.e. on bronchitis and pneumonia) had poor fit. Therefore, these items were excluded from this study. We found that antibiotic knowledge, as determined by three questions on appropriate antibiotic use, can be expressed as a unidimensional latent trait with an item response model showing adequate fit. Also, antibiotic knowledge was associated with other behaviors of antibiotic use both overall and across ethnic groups.

The psychometric study from **chapter 3** revealed that we did not have to use all 5 items for the IRT model presented in **chapter 2**. When repeating the analyses performed in **chapter 2** using the weighted sum score based on the 3-item questionnaire from **chapter 3**, the variables associated with antibiotic knowledge were the same. However, we did find that increased antibiotic knowledge, as expressed by an increased weighted sum score from the 3-item questionnaire, was associated with a lower number of antibiotic prescriptions in the year prior to the HELIUS study visit. Importantly, the differences in outcomes between ethnic groups also remained the same.

Kosiyaporn et al. published a systematic review on surveys of knowledge and awareness of antibiotic use and antimicrobial resistance(14). Themes commonly used in these studies were for instance frequency of using antibiotics, source of obtaining antibiotics, indication for use, instruction for use, type of antibiotic used, general knowledge, and awareness of not using antibiotics for the common cold or flu. The authors. observed key features in design and methodology of these surveys that could prove useful in the development of a tool to determine antibiotic knowledge. These were, rather general to all epidemiological studies, (I) a clear survey objective, (II) scientifically comprehensive sampling techniques ensuring representativeness, (III) strategies for recruitment of

samples and survey administration methods and (IV) reliable measurement to prevent non-sampling biases(14).

We recommend to develop and validate instruments measuring antibiotic knowledge that can be used for all ethnic groups. Firstly, such an instrument can be valuable to understand whether certain groups have limited understanding of antibiotics and could benefit from interventions to improve knowledge. Also, when a validated instrument is used in multiple studies worldwide, this improves comparability of studies into antibiotic knowledge within and between countries. Also, such an instrument could be used to measure the effect of public health campaigns on antibiotic knowledge.

The questionnaire we used **in chapter 3** was very short and only consisted of three adequate questions on appropriate antibiotic use, which made it easy to incorporate it in the lengthy HELIUS questionnaire. We suggest that future studies into antibiotic knowledge and reasons for differences in antibiotic knowledge between groups utilize a questionnaire fit to their study objective. The 3-item questionnaire we used could be used to determine antibiotic knowledge in large-scale studies in which measuring antibiotic knowledge is part of a broader array of research questions. But when performing a study solely targeted to measure antibiotic knowledge and related outcomes, we propose the usage of a more extensive questionnaire. The criterion validity of these instruments could be further assessed by questions on source of obtaining antibiotics, self-medication with antibiotics, awareness on the magnitude of the problem of antibiotic resistance, and faith in physicians' decisions (not) to prescribe antibiotics.

PREVALENCE OF NASAL MRSA AMONG UNDOCUMENTED MIGRANTS AND UNINSURED LEGAL RESIDENTS

A systematic review and meta-analysis on antimicrobial resistance among migrants in Europe showed a pooled prevalence of 7.8% for MRSA between 2000 and 2017(15). Migrants are therefore described as a group at risk for increased MRSA carriage(16-18). The systematic review and meta-analysis also showed a higher pooled prevalence of MRSA carriage or infection among specifically refugees and asylum seekers (8.2%) compared to other migrant groups (6.0%)(15). However, to date, no other studies have specifically investigated MRSA carriage among another demographic group, namely undocumented migrants and uninsured legal residents.

In **chapter 4,** we found that the prevalence for nasal MRSA carriage among undocumented migrants and uninsured legal residents in Amsterdam, the Netherlands, was

2.0% from October 2018 to October 2019. This prevalence was higher compared to the prevalence for the general Dutch population (<1% in 1999-2017)(19-21), but lower than found in the aforementioned systematic review among migrants in Europe (7.8%) and a study among asylum seekers in the Netherlands (9.7% in 2014-2015)(15, 16). It is important to investigate possible explanations for the differences in MRSA carriage between undocumented migrants and uninsured legal residents and other migrants and asylum seekers, since differences in screening policies or isolation upon hospital admission may be warranted.

Possibly, differences between asylum seekers and undocumented migrants and uninsured legal residents exist with respect to housing conditions, country of origin or socioeconomic status. In our study, we also hypothesized that the differences in MRSA prevalence between asylum seekers and undocumented migrants and uninsured legal residents could be due to their length of stay in the Netherlands. MRSA acquired from countries of origin or during travel to Europe may have cleared spontaneously in our study population by the time they were screened, since the median number of years since arrival in the Netherlands was 5 (inter quartile range 2-13). This hypothesis was in line with findings from another study that showed that asylum seekers living in the Netherlands for more than one year had a slightly lower prevalence of MRSA carriage compared to recently arrived migrants(22). The studies did not describe differences between high or low MRSA-endemic countries of origin.

It remains questionable whether additional screening for MRSA carriage among undocumented migrants and uninsured legal residents is warranted. Currently, in the Netherlands, all asylum seekers are screened for MRSA upon hospital admission or emergency care visit(18). Based on the relatively low prevalence of MRSA carriage found in our study, one could argue that screening of all undocumented migrants and uninsured legal residents would be unnecessary and that screening based on length of stay in the Netherlands would be more effective. In that case, travelers recently returning from highly endemic countries for MRSA should also be considered for screening, since multiple studies demonstrated the import of resistant bacteria through travelers(23-25). Currently such screening is only performed if a traveler was hospitalized abroad in high-endemic regions. A Swedish study even showed that out of all diagnosed MRSA cases in a three year period, 17% were imported through residents travelling abroad(26). It should however be noted that for our study the MRSA prevalence was based on estimates of relatively small sample sizes. In order to be able to make more sound recommendations, studies among broader migrant populations (both legal and illegal and both residing in urban and rural areas) are needed to further explain the underlying causes for the observed differences in MRSA carriage between different migrant populations. Also, since

the distribution of migrants and migration patterns are likely to change in the future, as they have in the past two decades, periodic surveillance among this heterogeneous population is warranted.

PREVALENCE OF ESBL-E AMONG MSM

It is widely known that ESBL-E carriage is associated with the use of antibiotics and can be transmitted to other individuals via close contact, for instance in the setting of travel to high prevalent areas or recent hospitalization(23, 27-33). Transmission could also be more proximal, namely during sexual contact. Indeed, between 2012 and 2017, several outbreak reports and one cross-sectional study suggested possible sexual transmission of ESBL-E among MSM(34-37). However, one study focused on the prevalence of gram-negative bacteria specifically among HIV-positive MSM(34) and the other studies focused on outbreaks of single bacteria(35, 36). All studies only retrospectively measured sexual behavior among a very small study population and were unable to differentiate multiple sexual behaviors and different types of sexual partners(34-36).

In **chapter 5**, we found that the ESBL-E prevalence of 16.3% among MSM was clearly higher compared to estimates (8.6%) for the general Dutch population in 2011(33). ESBL-E carriage was associated with a high number of sexual partners and practicing certain sexual behaviors with casual partner(s), especially those involving rimming, independent of recent use of antibiotics. These findings provide evidence that sexual transmission of ESBL-E is likely. In 2012, Borg et al. described that in-depth interviews with 7 MSM with confirmed *Shigella flexneri* infections revealed that all men reported sex with a casual contact in the week preceding illness(36). Subsequently, in 2016, Mook et al. described that of eight *Shigella sonnei* patients, seven completed detailed sexual history questions and reported to be MSM. Of these seven patients, six reported high risk sexual behaviors such as fisting, rimming and using sex toys with a partner(35). Taken together, MSM should be considered a group at risk of ESBL-E carriage. It is known that individuals with ESBL-E colonization are also at increased risk of ESBL-E infection(38). If ESBL-E infection occurs, effective treatment is often delayed, leading to increased mortality(39, 40).

We therefore believe that both MSM and medical professionals should be informed about this risk. For this purpose, it could be useful to approach sexual transmission of ESBL-E as we would sexually transmitted infections (STI) such as chlamydia and gonorrhea. By doing so, information on ESBL-E, the increased risk for MSM and the possibility for sexual transmission could be included in already existing STI information sources

and prevention campaigns (for instance through Soa Aids Nederland or the 'man tot man' website). Also, the association with increased numbers of sexual partners and particular sexual practices such as rimming (due to close oral-anal contact) should be stressed. Similarly, MSM should be informed that, even though the risk of ESBL-E infections is low in healthy individuals, ESBL-E carriage increases this risk of infection. Since carriage of ESBL-E does not warrant treatment, we do not advise periodic screening for ESBL-E among MSM.

We believe that raising ESBL-E awareness among MSM through popular online dating apps such as Grindr, PlanetRomeo and Scruff could also prove effective. These apps target MSM that are sexually active and have or are interested in (sex with) casual partners. A study from Barcelona demonstrated an 83% acceptability (proportion of users who responded favorably to a private message offering STI testing) and 73% effectiveness (those who attended an STI testing facility among those who were interested in attending) when piloting an STI intervention program aiming to increase STI testing through similar apps(41). Previously, studies from Mexico and the United States successfully piloted gamification to increase HIV and STI testing among MSM(42-44). Also, in 2016 a systematic review showed that gamification can indeed have a positive impact on health and wellbeing related behaviors(45). Gamification entails the use of elements and design of games in non-game related contexts(46). Since gamification has been used for STI prevention, we believe that gamification could also prove beneficial for informing MSM about ESBL-E and the possibility for sexual transmission. By competing in a game, MSM could compare scores on knowledge tests with other players and could earn medals/trophies/points that they can then use to upgrade their profile. The previously mentioned Mexican study showed that badges, points and prizes were perceived as enjoyable, exciting and motivating to compete(44). However, such a game should also include a certain action perspective. MSM could for instance be motivated to tell their health care provider that they might be at increased risk of ESBL-E carriage in case of infection or hospitalization. Healthcare providers could then consider adjusted antibiotic treatment. In order to realize such a game, the public health service should seek collaboration with companies that design the aforementioned apps for MSM. Also, the public health service of Twente, in collaboration with one of the ten regional care networks for ABR, previously developed a game, called 'infectionary', on infection prevention in nursing homes(47). Possibly, parts of this game could be used for the development of a game targeting MSM. When such a collaboration and gamification framework is in place, it could also be used for other STI prevention campaigns targeted at MSM.

Second, our study showed that 11% of participants was hospitalized in the preceding six months (reason for hospitalization was not obtained in the study). Transmission of ESBL-E between patients and healthcare providers can occur during hospital admission and therefore, physicians could consider different isolation precautions and empirical antibiotic treatment in case of severe sepsis in patients that are known MSM and taking sexual history (number of partners and types of practices) into account. For instance, patients that are known MSM and that have practiced sexual risk behavior could be isolated upon hospital admission until screening cultures for ESBL-E are negative, comparable with guidelines for risk groups for MRSA carriage. In case a known MSM that practices sexual risk behavior is treated for severe sepsis, based on our study and bearing possible ESBL-E colonization in mind, physicians could decide to treat the patient with a broader spectrum antibiotic. However, since sexual preference is not registered in electronic patient files, physicians could only base their decision to prescribe different antibiotics on their personal relationship with a patient. Also, we advise to motivate MSM at increased risk of ESBL-E carriage to actively express their sexual preferences and practices to their healthcare providers, for instance through the aforementioned game. We recommend that the regional care networks for antibiotic resistance could again play an important role in education of physicians on the increased prevalence of ESBL-E carriage among MSM and the association with sexual behavior. This Information should be included in the guideline for empirical antibacterial therapy of sepsis in adults as drafted by the Dutch Working Party on Antibiotic Policy (SWAB). It should be noted that the same results on increased ESBL-E carriage are expected for other individuals with high number of partners who are practicing certain sexual practices, such as sex workers or female sex workers. Further studies should confirm our findings in these groups.

PREVALENCE OF MULTIDRUG RESISTANT *ENTEROBACTERALES* AMONG RESIDENTS OF LONG-TERM CARE FACILITIES

Long-term care facilities (LTCF) provide care to mostly elderly patients. In this group, respiratory tract infections, urinary tract infections, and skin and soft tissue infections are common and can be caused by antibiotic resistant pathogens(48, 49).

In **chapter 6**, we found that the carriage rate of multidrug resistant (MDR) microorganisms among residents of LTCF in Amsterdam was nearly two times higher (18.2%) compared to estimates for the general population (8.6%) in 2011(33), but considerable differences between facilities existed (range 0-47%). These results were comparable with point prevalence studies reporting a multi-drug resistance organism (MDRO) carriage rate varying between 4% to 21% in Dutch long-term care facilities(50-53). High carriage

rates of MDR organisms could imply that residents of LTCF with a healthcare associated infections should be treated with second-line broad spectrum antibiotics(54). Increased use of these antibiotics could subsequently accelerate the emergence of resistance to these antibiotics. Also, if LTCF serve as a reservoir for MDR organisms, outbreaks with MDR bacteria can occur when residents of LTCF are referred to acute care facilities(55).

Since the Netherlands is a country with low rates of antibiotic resistance, an MDR organism carriage rate of 18% among residents of long term care facilities seems rather high. However, it should be noted that the prevalence we found was lower compared to the prevalence found in other European countries. A 2017 study from Italy showed a prevalence of colonization with ESBL-E of 57.3% among residents of LTCF(56). A review published in 2016 comparing colonization rates with MDR bacteria among LTCF in European countries found that rectal/perineal colonization with ESBL-E ranged from 3% in Sweden in 2008 to 55% in Ireland in 2012(57).

The consumption of antibiotics in LTCF stabilized in recent years (63.8 defined daily dose (DDD)/1,000 residents/day in 2011, 61.4 DDD/1,000 residents/day in 2018)(58). However, even though the prevalence of carriage of MDR organisms is considerably higher in LTCF compared to the general population, in general LTCF have a restrictive diagnostic policy and surveillance of antibiotic resistance is not systematically undertaken. Since 2018, updated guidelines for urinary tract infections and lower respiratory tract infections recommended diagnostics in LTCF in case of clinical infections(59, 60). Bearing in mind the huge variation in prevalence of MDR organisms between LTCF (varying from 0% to 47% in our study), adequate implementation of infection control practices is likely to differ between LTCF. Currently, multiple studies on MDR in LTCF are undertaken. These studies aim to identify structural differences between these centers and classify success factors in LTCF where the prevalence of MDR organisms is low.

In 2009, the RIVM launched a national sentinel surveillance network (SNIV) to monitor healthcare associated infections (HAI) in LTCF(61). The surveillance currently includes four surveillance modules: (I) HAI incidence module, (II) HAI prevalence module, (III) antibiotic use module, and (IV) in-depth surveillance module. Within these active sentinel surveillance modules, physicians and/or nurse practitioners weekly report the number of applicable cases and mortality through a web-based surveillance system, after which results are interpreted and processed by the RIVM(62). However, participating in SNIV is often perceived as labor intensive, especially for a sector in which the workload is already high. Therefore, we believe that a less labor intensive approach, for instance through sewage water measurements or through LQAS (lot quality assurance sampling – testing a small set of samples and determining whether they meet a predetermined

quality standard, for instance a predetermined resistance rate), would be beneficial for SNIV. Also, LTCF are obliged to provide the Dutch Healthcare Inspectorate with a lot of information and characteristics of their facility. Efforts should be made to transfer these data to SNIV in order that LTCF do not have to provide these data on multiple occasions. Missing critical data could then be collected separately for SNIV. Additionally, we would recommend SNIV to include an additional ABR module through sewage water measurements within LTCF. To enhance quick implementation of this module, we recommend SNIV to invite LTCF that already participate in one or more of the other modules to be included in this module. Ideally, LTCF across the country and with varying ABR prevalence rates would participate in order to shed light on best practices within LTCF. Insight into ABR prevalence and HAI incidence and prevalence could shed light on possibilities for improvement in infection control measures. Ultimately, decreasing ABR prevalence and HAI incidence and prevalence could improve resident health and decrease morbidity within the LTCF.

PERCEIVED BARRIERS AND ENABLERS FOR PREVENTING THE SPREAD OF CARBAPENEMASE PRODUCING GRAM-NEGATIVE BACTERIA DURING PATIENT TRANSFERS BETWEEN HEALTHCARE PROVIDERS

Several risk factors for the acquisition of carbapenemase producing gram-negative bacteria (CPB) among hospitalized patients, such as the use of medical devices and recent use of carbapenems, have been described(63, 64).

Additionally, as we described in **chapter 7**, risk of spread of CPB is also increased during the transfer of a CPB-positive patient between healthcare institutions. Our study illustrated that inadequate communication resulted in the risk of CPB spread being increased during the transfer of CPB-positive patients. A study from 2020 described that patient transfers influenced the prevalence of hospital-endemic pathogens and carbapenem-resistant pathogens(65). We found that healthcare providers were not used to caring for CPB-positive patients and that knowledge concerning CPB was low. The prevalence of CPB in the Netherlands is still low, but since infections with CPB are difficult to treat, the potential effect of these infections on morbidity and mortality is large.

Therefore, we believe that action should be taken whilst CPB prevalence remains low. Investing in prevention of transmission of CPB at this stage will hopefully result in the CPB prevalence remaining as low as it is today. We recommended that the information transfer concerning CPB-positive patients would be standardized to improve commu-

nication and that formal transmural agreements should be drafted in order to optimize communication concerning CPB-positive patients. Additionally, we recommended that healthcare providers' knowledge on CPB be enhanced by means of education (e.g. E-learning through a national website) and that dedicated employees for ABR should be appointed within healthcare institutions. These employees should for instance stimulate infection prevention measures, should make sure protocols are frequently updated and employees are regularly educated on ABR.

Once again, we believe that the regional care networks for ABR could play an important role. Drafting and strengthening transmural agreements concerning sharing information on outbreaks and carriage of multidrug resistant bacteria during patient transfers between healthcare institutions in the region is one of the main tasks of the regional care networks for ABR. Since these networks for ABR are currently working on transmural agreements for MDR organisms, we would advise the networks to ensure specific agreements for CPB in these transmural agreements. Also, the commitment of healthcare institutions to exchange CPB status and the necessary precautionary measures for individual patients before patient transfers take place and to be open about CPB status to involved professionals within their institutions should be included.

EFFECT OF COVID-19 ON ABR

The possible impact of the recent COVID-19 pandemic on antimicrobial resistance should be mentioned. During the past year, multiple experts stressed that the global COVID-19 pandemic presents several important consequences for ABR(66-70). Positively, additional infection control measures could decrease transmission of ABR bacteria within healthcare institutions and improved personal hygiene possibly decreased the spread of ABR bacteria within communities(70). On the other hand, studies showed that COVID-19 patients often receive antibiotics(69, 71, 72). Also, hospitalization leads to an increased risk for HAI and the transmission of ABR bacteria with subsequent increased antibiotic use(69, 73). Use and overuse of antibiotics are the main drivers of antibiotic resistance(74).

Recent studies from Germany, Italy and the United States have reported outbreaks or increased infections with MDR bacteria during the COVID-19 pandemic(75-78). On the contrary, several studies from France and Spain did not demonstrate increased infections with ABR bacteria(79, 80) and one Italian study showed a reduction in *Clostridioides difficile* infections in hospitalized patients(81).

We believe that for LTCF specifically, the COVID-19 pandemic may have a positive effect on ABR. The increased attention for infection prevention measures, social distancing and increased awareness among both healthcare providers, patients and visitors could result in decreasing transmission of ABR bacteria. Additionally, as also described in a previous study, we believe that due to COVID-19, fewer patients from long-term care facilities have been admitted to the hospital in the past year(68). This may have led to decreased transmission of ABR bacteria, including CPB, between long-term care facilities and hospitals.

CONCLUSIONS

The WHO's global action plan on antimicrobial resistance formulated 5 strategic objectives in the battle against ABR: 1) improve awareness and understanding of ABR through effective communication, education and training, 2) strengthen the knowledge and evidence base through surveillance and research, 3) reduce the incidence of infection through effective sanitation, hygiene and infection prevention measures, 4) optimize the use of antimicrobial medicines in human and animal health, and 5) develop the economic case for sustainable investment that takes into account of the needs of all countries, and increase investment in new medicines, diagnostic tools, vaccines and other inventions(82). Figure 1 shows where the chapters of this thesis fit in the objectives as posed by the WHO. For the Netherlands, there is still room for improvement on awareness and understanding of antimicrobial resistance, not only among the general public but also in specific groups including individuals from different ethnicities, MSM and undocumented migrants and uninsured legal residents, as the studies presented in this thesis have shown. As we illustrated, tailor-made efforts should be made to reach and educate these different groups. We also found evidence that surveillance of ABR throughout the country could be improved within (long-term) care institutions and among specific groups such as migrants. Also, infection control measures could be improved in certain LTCF. Insights in the prevalence and incidence of ABR in these groups could serve as input for tailored control measures and thereby potentially decrease ABR in these groups.

On a positive note, the incidence of ABR infections in the Netherlands is still low and efforts should be continued to remain among the countries with the lowest prevalence and incidence. Also, the restrictive use of antimicrobial agents in both human and animal health in the Netherlands contribute to the relatively low prevalence of ABR in the Netherlands today.

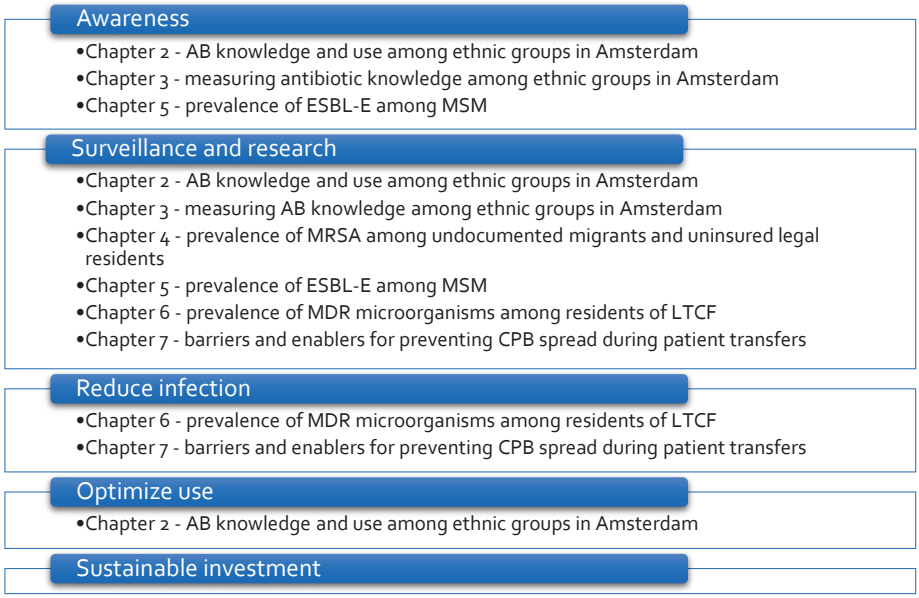

Figure 1. Chapters included in this thesis and their place within the strategic objectives described in the WHO global action plan on antimicrobial resistance
Abbreviations: AB – antibiotic; ESBL-E - extended spectrum β-lactamase producing Enterobacterales; MSM – men who have sex with men; MRSA – methicillin-resistant Staphylococcus aureus; MDR – multidrug resistant; LTCF = long-term care facilities; CPB - carbapenemase producing gram-negative bacteria

The studies presented in this thesis have provided insight into the occurrence of and risk factors for antibiotic resistance among specific demographic groups living in Amsterdam, the Netherlands, and have elaborated on the implications for public health. Furthermore, this thesis has provided several recommendations for actions that could help combat ABR in public health and within the cascade of care in the Netherlands. Specific recommendations include launching a differentiated prevention message to educate individuals from different ethnic groups on antibiotics and ABR, to inform sexually active MSM of their increased risk of ESBL-E carriage and the association with sexual behavior, to inform healthcare providers about ethnic differences in antibiotic use and about the increased prevalence of EBSL-E carriage among MSM and the association with sexual behavior, to study MRSA carriage among a wide array of migrants, to make formal transmural agreements on CPB and to improve and expand SNIV. We believe that the RIVM, regional ABR care networks, local public health services and professionals from hospitals, long term care facilities, primary care facilities, research institutes and non-governmental organizations (NGO's) should join forces to help combat ABR in the Netherlands.

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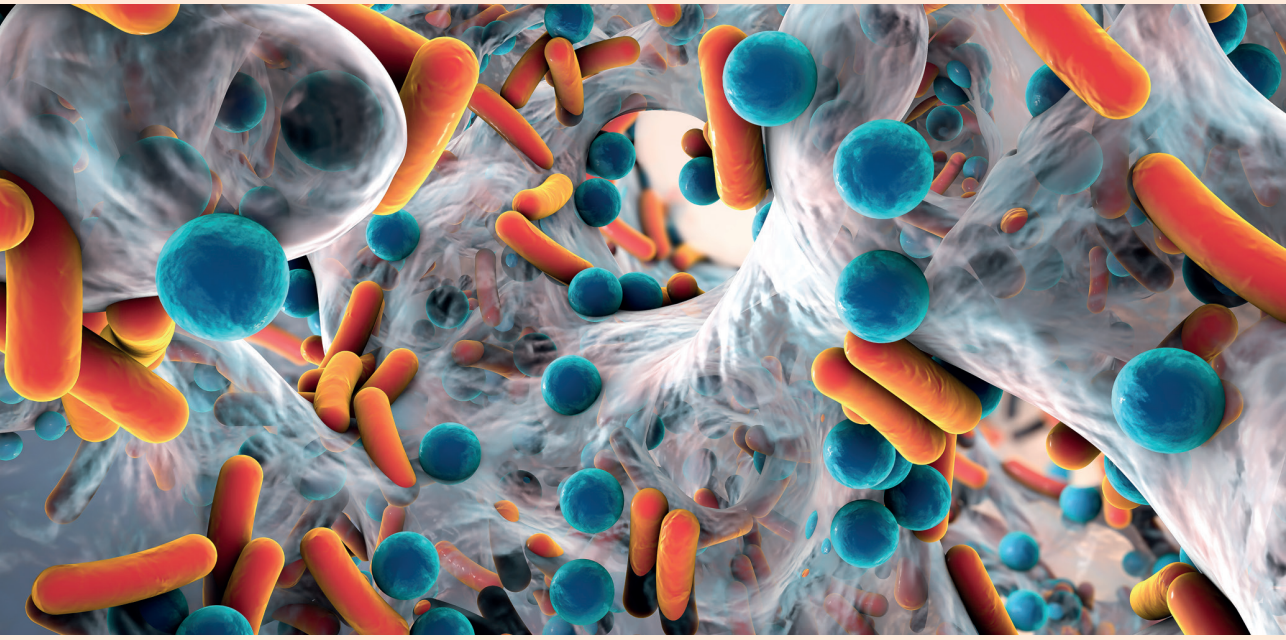

Appendix

SUMMARY – ANTIBIOTIC RESISTANCE IN SPECIFIC SOCIODEMOGRAPHIC GROUPS: IMPLICATIONS FOR PUBLIC HEALTH

The studies included in this thesis aimed to describe the occurrence of and associated factors with antibiotic resistance among specific sociodemographic groups living in Amsterdam, the Netherlands. The results from these studies allow a more specific understanding of the public health needs for these groups. **Chapter 1** provides a general introduction on the topic and briefly describes the antibiotic era, emergence of antibiotic resistance, disease burden of antibiotic resistant infections, drivers of antibiotic resistance and the current epidemiology and national approach of the Netherlands. The multidrug-resistant organisms that were covered in this thesis are also briefly described. Finally, **chapter 1** gives an overview of the proceeding chapters of this thesis.

In **part I** (chapters 2- 4) of this thesis, antibiotic knowledge, antibiotic use and prevalence of methicillin-resistant *Stapylococcus aureus* (MRSA) among different migrant groups living in Amsterdam, the Netherlands are studied. In **chapter 2**, we studied antibiotic knowledge (as measured by a 5-item questionnaire on appropriate antibiotic use) and antibiotic use (as obtained from health insurance data) among individuals from six ethnic groups (South-Asian Surinamese, African Surinamese, Ghanaian, Turkish, Moroccan and Dutch). We found that all ethnic minority groups had lower antibiotic knowledge compared to the Dutch group, while some ethnic groups more frequently used antibiotics. Furthermore, we established in our study that level of antibiotic knowledge was not associated with antibiotic use. In **chapter 3**, we further studied the concept of antibiotic knowledge and analyzed the psychometric properties of the items used to measure antibiotic knowledge, particularly between ethnic groups, in **chapter 2**. From the original 5-item questionnaire, we found that antibiotic knowledge can be measured by 3 items on appropriate antibiotic use across all ethnicities in large-scale studies in which measuring antibiotic knowledge is part of a broader array of questions besides antibiotic resistance. In **chapter 4**, we determined that the prevalence of nasal MRSA carriage was 2.0% among undocumented migrants and uninsured legal residents in Amsterdam, the Netherlands in 2018-2019. This prevalence was higher compared to the general Dutch population (<1% in 1999-2017), but lower compared to other European studies among migrants (6.0-8.2% in 2000-2017).

Part II (chapters 5 and 6) of this thesis focuses on the prevalence of extended spectrum β-lactamase producing *Enterobacterales* (ESBL-E) among specific groups in Amsterdam, the Netherlands. In **chapter 5**, we describe that the prevalence of rectal ESBL-E carriage was 16.3% among men who have sex with men, which is considerably higher compared

to estimates of the general population in 2011 (8.6%). Additionally, we determined that ESBL-E carriage was associated with a higher number of sexual partners and practicing certain sexual behaviors (especially rimming) with casual partners. In **chapter 6**, we established that the prevalence of multidrug resistant microorganisms among residents of long-term care facilities was approximately two times higher (18.2%) compared to the general population (8.6%), but that there were considerable differences between facilities (range 0-47%).

In **part III** (chapter 7) of this thesis, we describe the perceived barriers and facilitating factors for preventing the spread of carbapenemase-producing gram-negative bacteria (CPB) during patient within and between healthcare facilities in the Dutch provinces of Noord-Holland and Flevoland. **Chapter 7** illustrates that inadequate communication resulted in a higher risk of CPB spread during the transfer of CPB-positive patients. Based on qualitative interviews, healthcare providers were not accustomed to caring for CPB-positive patients and knowledge concerning CPB was low.

In **chapter 8**, the relevance of the findings from this thesis is discussed and placed into context with the most recent literature, followed by recommendations for future research and public health interventions. Furthermore, this chapter describes the possible effects of the current COVID-19 pandemic on antibiotic resistance. Finally, we describe how the studies from this thesis fit into the 5 strategic objectives of the World Health Organization's global action plan on antimicrobial resistance is broached. The studies in this thesis showed that there is still room for improvement on awareness and understanding of antimicrobial resistance in the Netherlands among specific groups including individuals from different ethnicities, MSM and undocumented migrants and uninsured legal residents. As we illustrated, efforts should be made to reach, serve and educate these different groups. Also, surveillance of ABR in long term care facilities in the Netherlands could be improved. The incidence and prevalence of ABR infections in the Netherlands is still low and efforts should be continued to remain among the lowest-ranking countries. We therefore recommend the following:

- Launching a differentiated prevention message to educate individuals from different ethnic groups on antibiotics and ABR.
- To inform MSM of their possible increased risk of ESBL-E carriage and the association with sexual behavior.
- To inform healthcare providers about ethnic differences in antibiotic use and about the increased prevalence of EBSL-E carriage among MSM and the association with sexual history (number of partners and types of practices).
- To study MRSA carriage among a wide array of migrants.
- To improve and expand SNIV with a less labor intensive approach.

- To make transmural agreements on transferring CPB-positive patients between healthcare institutions.

SAMENVATTING – ANTIBIOTICARESISTENTIE ONDER SPEFICIEKE SOCIODEMOGRAFISCHE GROEPEN: IMPLICATIES VOOR DE PUBLIEKE GEZONDHEID

De studies in dit proefschrift hadden tot doel om het vóórkomen van en factoren geassocieerd met antibioticaresistentie (ABR) onder specifieke sociodemografische groepen in Amsterdam te beschrijven. De resultaten van deze studies bieden een beter inzicht in specifieke behoeften voor deze groepen vanuit de publieke gezondheid . **Hoofdstuk 1** biedt een algemene introductie op het onderwerp van dit proefschrift en beschrijft kort de opkomst van het antibioticatijdperk, het ontstaan van ABR, de ziektelast van antibioticaresistente infecties, oorzaken van ABR en de huidige epidemiologie en nationale aanpak van ABR in Nederland. Tevens worden de multiresistente organismen die in dit proefschrift zijn behandeld kort beschreven. Ten slotte is in **hoofdstuk 1** een overzicht van de overige hoofstukken in dit proefschrift opgenomen.

In **deel I** (hoofdstukken 2-4) van dit proefschrift zijn antibioticakennis, antibioticagebruik en de prevalentie van methicilline-resistente *Staphylococcus aureus* (MRSA) binnen verschillende migrantengroepen in Amsterdam bestudeerd. In **hoofdstuk 2** zijn antibioticakennis (gemeten met een vragenlijst waarin vijf vragen over correct antibioticagebruik waren opgenomen) en antibioticagebruik (verkregen door middel van koppeling met verzekeringsdata) onderzocht bij Amsterdammers uit zes etnische groepen (van Hindoestaans-Surinaamse, Afrikaans-Surinaamse, Ghanese, Turkse, Marokkaanse en Nederlandse herkomst). Onze studie toonde aan dat alle etnische minderheidsgroepen minder antibioticakennis hadden dan de Nederlandse groep. Tevens bleek het antibioticagebruik in sommige etnische groepen hoger dan in andere groepen. Ten slotte toonde onze studie aan dat antibioticakennis niet geassocieerd was met antibioticagebruik. In **hoofdstuk 3** hebben we het concept antibioticakennis verder onderzocht en hebben we de psychometrische aspecten (in het bijzonder tussen etnische groepen) van de vragen die in **hoofdstuk** 2 zijn gebruikt om antibioticakennis te meten verder geanalyseerd. Onze studie liet zien dat antibioticakennis in grootschalige studies waarin het meten van antibioticakennis onderdeel is van een bredere pakket aan vragen, bij alle etnische groepen gemeten kan worden met drie van de oorspronkelijke vijf vragen over correct antibioticagebruik. In **hoofdstuk 4** stelden we vast dat de prevalentie van dragerschap van MRSA in de neus bij ongedocumenteerde personen met een migratiegeschiedenis en onverzekerde legale inwoners van Amsterdam in 2018-2019 2,0% was. Deze prevalentie was hoger dan die onder de algemene Nederlandse bevolking (<1% tussen 1999 en 2017), maar lager dan de prevalenties die in andere Europese studies onder personen met een migratiegeschiedenis werden gevonden (6,0-8,2% tussen 2000-2017).

Deel II (hoofdstukken 5 en 6) van dit proefschrift richt zich op de prevalentie van extended-spectrum β-lactamase producerende *Enterobacterales* (ESBL-E) binnen specifieke groepen in Amsterdam. In **hoofdstuk 5** beschrijven we dat de prevalentie van rectaal dragerschap van ESBL-E 16,3% was bij mannen die seks hebben met mannen (MSM). Deze prevalentie is aanzienlijk verhoogd vergeleken met schattingen van de prevalentie onder de algemene bevolking in 2011 (8,6%). Tevens vonden we dat er een associatie bestaat tussen ESBL-E dragerschap en een hoger aantal sekspartners en bepaalde seksuele handelingen (met name rimmen) met losse sekspartners. In **hoofdstuk 6** stellen we vast dat de prevalentie van multiresistente micro-organismen bij inwoners van verzorg- en verpleeghuizen ongeveer twee keer hoger was (18,2%) dan onder de algemene bevolking (8,6%), maar dat er aanzienlijke verschillen bestonden tussen instellingen (range 0-47%).

In **deel III** (hoofdstuk 7) van dit proefschrift zijn barrières en faciliterende factoren bij zorgverleners in Noord-Holland en Flevoland voor het voorkómen van de verspreiding van carbapenemase-producerende gramnegatieve bacteriën (CPB) tijdens het overplaatsen van patiënten binnen en tussen zorginstellingen beschreven. **Hoofdstuk 7** laat zien dat inadequate communicatie resulteert in een verhoogd risico op verspreiding van CPB tijdens overplaatsingen van CPB-positieve patiënten. Op basis van kwalitatieve interviews hebben we gevonden dat zorgverleners niet gewend waren om te zorgen voor CPB-positieve patiënten en dat kennis over CPB onder zorgverleners laag was.

In **hoofdstuk 8** wordt de relevantie van de bevindingen van dit proefschrift besproken en worden deze bevindingen in context geplaatst van de meest recente literatuur. Ook worden aanbevelingen gedaan voor verder onderzoek en publieke gezondheidsinterventies. Tevens omschrijft dit hoofdstuk de mogelijke impact van de huidige COVID-19 pandemie op ABR en wordt ten slotte beschreven hoe de studies in dit proefschrift passen binnen de 5 strategische doelen van het wereldwijde actieplan ABR van de WHO.

De studies in dit proefschrift laten zien dat er nog altijd ruimte voor verbetering is op het gebied van bewustwording en begrip omtrent ABR in Nederland onder specifieke etnische groepen, MSM en ongedocumenteerde personen met een migratieachtergrond en onverzekerde legale inwoners. Zoals dit proefschrift illustreert, is het belangrijk de inspanningen om deze verschillende groepen te bereiken, te bedienen en bij te scholen worden voortgezet. Tevens kan surveillance van ABR in Nederlandse verpleeghuizen worden verbeterd. Nederland is één van de landen met de laagste incidentie en prevalentie van antibioticaresistente infecties wereldwijd en de huidige aanpak moet worden voortgezet om dat zo te houden, maar deze aanpak kan op sommige punten nog wel verbeterd worden. Daarom worden in hoofdstuk 8 de volgende aanbevelingen gedaan:

- Het opstellen van een gedifferentieerde preventieboodschap om verschillende etnische groepen bij te scholen over antibiotica en ABR.
- Het informeren van MSM over het mogelijk verhoogde ESBL-E dragerschap en de associatie van dragerschap met seksueel gedrag (aantal sekspartners en bepaalde seksuele handelingen).
- Het informeren van zorgverleners over etnische verschillen in antibioticagebruik en de verhoogde prevalentie van ESBL-E dragerschap onder MSM en de associatie met seksueel gedrag.
- Het bestuderen van MRSA dragerschap onder een brede groep van personen met een migratiegeschiedenis.
- Het verbeteren en uitbreiden van het Surveillance Netwerk Infectieziekten in Verpleeghuizen (SNIV) middels een minder arbeidsintensieve werkwijze.
- Het opstellen van transmurale werkafspraken over de overplaatsing van CPB-positieve patiënten tussen zorginstellingen.

PORTFOLIO

PhD training	Year	Workload (ECTS)
AMC Graduate School, Amsterdam, the Netherlands		
- Infectious Diseases	2017	1.3
- Basic Course Qualitative Health Research	2018	1.9
- Endnote	2018	0.1
- BROK (Basic course on Regulations and Organisation for clinical investigators)	2018	1.5
- Clinical Epidemiology: Systematic Reviews	2019	0.7
- Project Management	2019	0.6
Netherlands Institute for Health Sciences (NIHES), Erasmus University Rotterdam, the Netherlands		
- Using R for Statistics in Medical Research	2019	1.4
GGD Amsterdam, the Netherlands		
- Weekly PhD educational hour (seminars, journal club, peer education, epidemiology class)	2017-2020	14.4
Seminars, workshops		
- Netwerken in ABR-surveillance RIVM, Bilthoven, the Netherlands	2017	0.1
- Meet & Great (gezondheidsprofessionals sekswerk), Utrecht, the Netherlands	2017	0.1
- Minisymposium Infectieziektendiagnostiek GGD, Amsterdam, the Netherlands	2017	0.1
- RAC scholing “BRMO-clusters in het publieke domein” RIVM, Bilthoven, the Netherlands	2017	0.1
- ABR Symposium RIVM, Bilthoven, the Netherlands	2018	0.1
- ABR in de ouderenzorg RIVM/Vilans, Bilthoven, the Netherlands	2018	0.1
- AMPHI Outbreak Workshop, Radboud UMC, Nijmegen, the Netherlands	2018	0.1
- GGD Jaarseminar afdeling onderzoek, Amsterdam, the Netherlands	2018, 2020	0.2
- Annual Public Health meeting Amsterdam UMC, Amsterdam, the Netherlands	2019	0.1
- GGD Onderzoeksdag, Amsterdam, the Netherlands	2019, 2021	0.2

Presentations		
- "Sexually transmitted infections among female sex workers in Amsterdam between 2011 and 2016: does risk vary by work location?" (poster presentation at the European Congress of Clinical Microbiology and Infectious Diseases (ECCMID), Madrid, Spain)	2018	0.5
- "Knowledge and use of antibiotics in six ethnic groups. The HELIUS study" (poster presentation at the Annual Public Health Meeting Amsterdam UMC, Amsterdam, the Netherlands, and at the European Congress of Clinical Microbiology and Infectious Diseases (ECCMID), Amsterdam, the Netherlands)	2019	1.0
(Inter)national conferences		
- European Scientific Conference on Applied Infectious Disease Epidemiology, Stockholm, Sweden	2017	1.3
- Infectious diseases symposium, Amsterdam, the Netherlands	2017-2019	0.9
- European Congress of Clinical Microbiology and Infectious Diseases (ECCMID), Madrid, Spain	2018	1.3
- Dutch National STI, HIV and sex conference, Amsterdam, the Netherlands	2018	0.3
- Dutch National Hepatitis Conference, Utrecht, the Netherlands	2018	0.3
- 22nd International AIDS Conference, Amsterdam, the Netherlands	2018	1.3
- European Congress of Clinical Microbiology and Infectious Diseases (ECCMID), Amsterdam, the Netherlands	2019	1.3
Coordinating tasks		
- Epidemiologist for Antibiotic Resistance Care Networks	2017	18
- PhD Tea (discussing methodological issues and reviewing papers, GGD Amsterdam)	2018-2019	0.6

Teaching	**Year**	**Workload (ECTS)**
Supervising		
- Sally Eskander *"Risk group for HBV, HCV, HIV and MRSA out of view!?"* (Msc Medicine, University of Amsterdam)	2018	0.8
- Savanna Letteboer *"Hepatitis B virus, hepatitis C virus and HIV infection in undocumented migrants in Amsterdam"* (Msc Medicine, University of Amsterdam)	2019	0.8
- Mustafa Kahveci *"Hepatitis B virus, hepatitis C virus and HIV infection in undocumented migrants in Amsterdam"* (Msc Medicine, University of Amsterdam)	2019	0.8
- Marijne Zandbelt *"Screening op tuberculose-infectie bij risicogroepen in Amsterdam, evaluatie-onderzoek van een nieuwe interventie"* (MD, department of tuberculosis, GGD Amsterdam)	2019	0.8
- Floor Steenwinkel *"Methicillin resistant Staphylococcus aureus (MRSA): a cross-sectional study in undocumented migrants in Amsterdam"* (BSc Medicine, University of Amsterdam)	2019	0.8
- Frans Thomas *"Perceived barriers and facilitators to implement screening advice by general practitioners for hepatitis B and C: A qualitative study"* (Msc Medicine, University of Amsterdam)	2019	1.7
	Total	55.6

LIST OF PUBLICATIONS

Publications included in this thesis

Van Dulm E, Tholen ATR, Pettersson A, van Rooijen MS, Willemsen I, Molenaar P, Damen M, Gruteke P, Oostvogel P, Kuijper EJ, Hertogh CMPM, Vandenbroucke-Grauls CMJE, Scholing M. High prevalence of multidrug resistant *Enterobacteriaceae* among residents of long term care facilities in Amsterdam, the Netherlands. **PLoS ONE**. 2019 Sep 12;14(9).

Schuts EC*, van Dulm E*, Boyd A, Snijder MB, Geerlings SE, Prins M, Prins JM. Knowledge and use of antibiotics in six ethnic groups: the HELIUS study. **Antimicrob Resist Infect Control**. 2019 Dec 6;8:200.

Van Dulm E, Prins M, Prins JM, Galenkamp H, Zwinderman AH, Boyd A. Measuring antibiotic knowledge using a three-item questionnaire in different ethnic groups of the general population: a psychometric analysis in the Helius Study. **Submitted**.

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Eline van Dulm-Freriks was born on December 19, 1989 in Nijmegen, the Netherlands. She grew up in Amersfoort and graduated from 't Hooghe Landt college in 2007. Following high school, she started her studies in Behavior and Society (Gedrag en Samenleving) at the University of Amsterdam (UvA). One year after, she transferred to a bachelor's degree program at the Hotel Management School Maastricht. Her studies there allowed her to complete an internship in Aruba in 2010 and a graduation project in South Africa in 2012, after which she obtained her diploma in 2012. In 2013, she enrolled in the Health Sciences program at the VU University of Amsterdam. Her bachelor's thesis was nominated for the VU thesis prize in 2016, which she won for best thesis pitch. In 2017, she obtained her masters degree in Infectious Diseases and Public Health cum laude at VU University of Amsterdam. She started her PhD in August 2017 at the Department of Infectious Diseases Research and Prevention at the Public Health Service of Amsterdam, under the supervision of Prof. Maria Prins, Prof. Jan Prins (Department of Internal Medicine, Amsterdam UMC, location AMC), Dr. Yvonne van Duijnhoven and Dr. Anders Boyd. The results of this work are presented in this thesis. In August 2020, Eline started working as manager for the Covid-19 contact tracing department of the Public Health Service of Amsterdam.

In 2021, Eline is living in Zeist with her husband, Onko, and their son, Hugo.

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