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Microbiological Surveillance in Primary Health Care

New Aspects of Antimicrobial Resistance and Molecular Epidemiology in an Ageing Population

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To Elisabeth

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Abstract

Background

The inexorable rise in antimicrobial resistance (AMR) interferes with the goals of health care services around the world, given how critical the antibacterials are in making infections treatable and surgical procedures doable. Nursing homes residents have been identified as a reservoir for AMR, possibly due to the combination of being physically and mentally frail, frequently treated with antibacterials, and frequently moved between nursing home and hospital. Microbiological surveillance is a key countermeasure against further AMR development. Yet, surveillance data is easily biased due to precision problems regarding how the data is collected and evaluated.

Methods

Beginning in 2008, we launched two programmes (“SHADES” and “MIDIO”) aimed to gathering AMR data in a systematic fashion from elderly nursing home residents and elderly people living in their own place of residence. In doing so, we focused on colonizing strains of the two most important nosocomial infectious agents, *Staphylococcus aureus (S. aureus)* and *Escherichia coli (E. coli)*. The bacteria were collected from multiple body sites and analysed with respect to antimicrobial susceptibility and genetic diversity.

Results

Active surveillance of AMR showed that (i) a *S. aureus* isolate could be retrieved from 1 in every 2 individuals given a single round of sampling, but aggregating several rounds of sampling, this figure might reach 7 in every 10 individuals, (ii) an *E. coli* isolate could be retrieved from 4 in every 5 individuals, (iii) the overall prevalence of AMR was favourable when compared to the situation in many other countries, (iv) the genetic diversity of *S. aureus* was generally high and provided only limited evidence of clonal expansion or contraction, and (v) diabetes mellitus was one of very few patient-level factors to show an association with the degree of genetic diversity in *S. aureus*.

Conclusions

The prevalence of colonization with *S. aureus* and *E. coli* was somewhat higher than expected, but the degree of AMR was very low. The genetic diversity of *S. aureus* was generally high. Diabetes mellitus emerged as the only patient-level factor associated with a higher degree of genetic diversity in *S. aureus*.

List of Papers

This thesis is based on the following original papers, which are referred to in the text by their Roman numerals.

I. Olofsson M, Lindgren PE, Östgren CJ, Midlöv P, Mölstad S. Colonization with Staphylococcus aureus in Swedish nursing homes: A cross-sectional study. *Scand J Infect Dis*. 2012;44(1):3-8.

II. Olofsson M, Toepfer M, Östgren CJ, Midlöv P, Matussek A, Lindgren PE, Mölstad S. Low level of antimicrobial resistance in Escherichia coli among Swedish nursing home residents. *Scand J Infect Dis*. 2013;45(2):117-23.

III. Stark L, Olofsson M, Löfgren S, Mölstad S, Lindgren PE, Matussek A. Prevalence and molecular epidemiology of Staphylococcus aureus in Swedish nursing homes--as revealed in the SHADES study. *Epidemiol Infect*. 2014;142(6):1310-6.

IV. Olofsson M, Löfgren S, Matussek A, Lindgren PE, Mölstad S, Östgren CJ. Molecular epidemiology of Staphylococcus aureus in an elderly Swedish population: A cross-sectional study on the impact of housing and diabetes mellitus. Unpublished manuscript.

Abbreviations and Acronyms

AMR	Antimicrobial resistance
BMI	Body mass index
DNA	Deoxyribonucleic acid
E. coli	Escherichia coli
ESBL	Extended-spectrum beta-lactamase producers
FORSS	Medical Research Council of Southeast Sweden
HIV	Human immunodeficiency virus
MIDIO	Microbiological Diversity in Ödeshög
MRSA	Methicillin-resistant Staphylococcus aureus
NordicAST	Nordic Committee on Antimicrobial Susceptibility Testing
OR	Odds ratio
S. aureus	Staphylococcus aureus
SHADES	Study of Health and Drugs in the Elderly
spa	Staphylococcus protein A gene
WHO	World Health Organization

Introduction

Rising Antimicrobial Resistance

Caring for Elderly Nursing Home Residents

WITHIN THE SWEDISH HEALTH CARE ORGANIZATION, elderly nursing home residents are usually given medical attention by family physicians who are employed in the public primary health care services. In our experience, the work done by family physicians at nursing homes is not unlike the work done by senior physicians in hospitals—the operation is centred around a scheme of recurring rounds, during which the medical staff meet, discuss, and solve problems from start to finish. In recent years, there has been much debate on the ongoing rise in antimicrobial resistance and the ways it might interfere with—or even disrupt—the goals of health care services, given how critical the antibacterials are in making infections treatable and surgical procedures doable. Until now, the Scandinavian countries have been spared the most agonizing aspects of antimicrobial resistance, which would be infections that are completely insensitive to every available antibacterial compound. However, the incidence of multi-drug-resistant extended-spectrum beta-lactamase producing bacteria has been growing at an extreme rate also in Sweden. Many cases have reached the nursing homes, forcing family physicians to pay special attention to hospital quality hygiene, bacterial sampling, and a tactical choice of antibacterial treatment.

Global Mega-Trend and Global Health Threat

The inexorable rise in antimicrobial resistance (AMR) is a global mega-trend that permeates every society, every health care organization, and down to every infectious agent [1 - 9]. The mechanisms behind the trend are complicated, but the human factor plays an important part, given the less-than-ideal way in which antibacterial compounds are being used [10 - 13]. The World Health Organization (WHO) recognizes that the rising AMR "threatens the effective prevention and treatment of an ever-increasing range of infections caused by bacteria, parasites, viruses and fungi." WHO also recognises that

the rising AMR leads to an increase in the infectious disease burden, given that infections caused by multi-drug-resistant organisms result in lengthier hospital stays and a need for more specialized health care [14 - 17]. An increase in mortality due to infections with multi-drug-resistant organisms has also been seen [18, 19].

"Giants" of Infectious Disease Burden

The three "giants" inflicting life-threatening disease in millions of people worldwide are *Mycobacterium tuberculosis* causing tuberculosis, human immunodeficiency virus (HIV) causing the acquired immunodeficiency syndrome, and the different species of *Plasmodium* causing malaria. All these infectious diseases have been associated with a rise in AMR [20 - 28]. For example, 3.3 % of new tuberculosis cases worldwide are now connected with strains of *Mycobacterium tuberculosis* which are resistant to both of the two most important tuberculostatic compounds in existence, rifampicin and isoniazid [29, 30]. A definite percentage of AMR in both HIV and malaria is not as easy to attain, given that these two infectious agents cannot be cultivated in the laboratory to create traditional antibiograms (or anti-microbial susceptibility diagrams). As a result, the extent of AMR in HIV and malaria has to be based on other types of statistics, e.g. the registered proportion of treatment failures in a particular geographical area [31, 32].

Further Infections with a High Disease Burden

The rising AMR in tuberculosis, HIV and malaria might signify the world's greatest challenge with respect to biological complexity and infectious disease burden. Even so, a diverse array of other infections also adds to the global health threat. In the sphere of endemic infections (i.e. infections that keep circulating among susceptible individuals, rather than forming a definable and long-standing reservoir), one example is *Neisseria gonorrhoeae*, a cause of gonorrhoea [33, 34]. In the sphere of opportunistic infections (i.e. the infectious agents colonize the host without inflicting any damage, yet remains capable of causing harm to the host in response to certain stimuli), one example is *Escherichia coli* which causes dysentery [35, 36]. Specifically, *Neisseria gonorrhoeae* reached a maximum of 36 % resistance to third-generation cephalosporins in clinical samples collected in the WHO European region in 2015, while *Escherichia coli* reached a portentous maximum of 82 % in certain parts of the same region [37].

Nosocomial Infections

Nosocomial Infections Are Diverse

The repercussions of the rising AMR affect every health care organization, not only because the treatment armamentarium is losing efficacy but also because the hospital environment is itself vulnerable to microbiological interference, and especially to opportunistic infectious agents. When an opportunistic infection occurs in connection with caring for a sick person, the infection is known as a nosocomial infection [38, 39]. The mechanisms underlying the different nosocomial infections cover many aspects of everyday life in the hospital—anything from providing unintentional transmission opportunities by bringing patients close to one another to breaching natural infection barriers by introducing catheters through the skin [40]. Nosocomial infections have been associated with a diverse array of infectious agents, e.g. *Staphylococcus aureus*, *Escherichia coli*, *Clostridium difficile*, and *Pseudomonas aeruginosa* [35 - 47].

Important Nosocomial Infectious Agents

Given the diversity and dissimilarity of the nosocomial infections, rating different infections according to seriousness is not a straightforward task [48, 49]. From our standpoint, *Staphylococcus aureus (S. aureus)* and *Escherichia coli (E. coli)* might be regarded as the world's most important nosocomial infectious agents. The justification for our claim lies in the poignant combination of pathogenic versatility and powerful mechanisms to accelerate AMR development. Both *S. aureus* and *E. coli* are ubiquitous infectious agents and widespread commensals with a capacity for clinically quiet, long-lasting propagation in the host. Furthermore, both species have the pathogenic capacity to invade the bloodstream causing septicaemia as well as additional pathogenic mechanisms that cover multiple organ systems [50 - 56]. For instance, *S. aureus* might infect the heart valves (endocarditis), the bone (osteomyelitis), and the lungs (pneumonia) [57]. An *E. coli* infection might involve the urinary bladder (urinary tract infection), the kidneys (pyelonephritis), and the gastrointestinal tract (dysentery) [58]. Both *S. aureus* and *E. coli* are prone to accelerated AMR development, given the widespread occurrence of genetic transferral events that confer entire sets of interconnected resistance mechanisms. At one stroke the strain goes from susceptible to multi-drug-resistant, as opposed to the gradual, one-step-at-a-time AMR

development seen in other bacterial species. Many *S. aureus* strains have acquired resistance to methicillin (methicillin-resistant *S. aureus*, MRSA), while many *E. coli* strains have acquired resistance to beta-lactam antimicrobial compounds (extended-spectrum beta-lactamase producers, ESBL) [59 - 64].

Burden of MRSA and ESBL in Europe

The European Centre for Disease Prevention and Control reported in 2014 that the proportion of invasive MRSA (i.e. MRSA isolates recovered from the blood stream) reached 17.4 % among European surveillance samples. The corresponding percentage in Sweden was 1.0 %. A definite proportion of invasive ESBL isolates cannot be given as easily, however, bearing in mind that ESBL-producing bacterial strains are not always *E. coli* (other types of Gram-negative bacteria can also have ESBL-producing strains). Employing a closely related estimate, the extent of AMR might be expressed as the proportion of invasive *E. coli* isolates resistant to third-generation cephalosporins. In 2014, this proportion reached 12.0 % in European surveillance samples. The percentage in Sweden was 5.6 % [65].

Surveillance Is a Key Countermeasure

Surveillance of Antimicrobial Resistance

In addition to the work done in the areas of sanitation and clean drinking water, the principal countermeasures against the rising AMR have been summarized by the WHO in a global action plan [6, 66]. The five main objectives listed in the action plan are (i) awareness and education, (ii) surveillance of AMR, (iii) infection prevention and control, (iv) optimal use of antimicrobial medicines in human and animal health, and (v) research, development and investment. The number two objective (surveillance of AMR) serves to monitor AMR development by collecting statistics based on clinical microbiological samples. This process recently became global as the WHO implemented the WHO Global Antimicrobial Resistance Surveillance System in an effort to combine the statistics from all major national and regional surveillance consortia in the world [67].

Precision Problems and Denominator Effect

Although a key strategy in combating AMR, surveillance done by compiling statistics from a host of different data sources might be biased. For example, the statistics might be afflicted by resource problems in low-income areas of the world, standardization problems due to the complexity of infectious medicine, and a particular statistical problem known as the "denominator effect" [37, 68, 69]. The denominator effect comes into play when the documentation regarding the outcome of empirical treatment (i.e. when treatment with antibacterials is given without precise knowledge of the infectious agent that caused the disease) is of questionable quality. An unreliable account of successful outcomes translates into a reciprocal over-estimation of unsuccessful treatments, given that the latter generate more clinical samples and run together with a higher risk of AMR. To some extent, precision problems can be addressed using active surveillance of AMR, in which samples are collected systematically from defined populations and in conjunction with information on antibacterial treatment [70, 71].

Economizing the Need for Accurate Estimates

Working Models and Proxies

The obvious drawback of active surveillance of AMR is the high cost, which makes comprehensive screening strategies unrealistic. However, the process might be economized by letting theoretically trustworthy working models and proxies act as a centre point for extrapolation. In line with our previous claim that *S. aureus* and *E. coli* might be regarded as the two most important nosocomial infectious agents, the same two bacterial species might also be regarded as essential indicator bacteria, given that expanded knowledge on the duality of one essential Gram-positive and one essential Gram-negative organism might symbolize a "greater whole." Extending the argument, a known reservoir for essential indicator bacteria might be regarded as a representation of a wider epidemiological perspective. The scientific interest in nursing homes as reservoirs for AMR bacteria has escalated, possibly because of the poignant combination of elderly people being crowded, physically and mentally frail, frequently treated with antibacterials, and frequently moved between nursing home and hospital [72 - 88].

Active Surveillance in Primary Health Care

Drawing from the credibility and conceptual trustworthiness of indicator bacteria, and reservoirs for such bacteria as working models and proxies, our research has been aimed to exploring active surveillance of AMR in defined populations of elderly nursing home residents. The *SHADES* and *MIDIO* Programmes have been a part of this aim. Given that some of us are working as family physicians, defining the populations has run together with the context of primary health care.

Aims

The general aim was to expand the knowledge of the human-bacterial ecology by applying active surveillance of AMR to defined populations of elderly nursing home residents, and to elderly people living in their own place of residence in the geographical area surrounding a nursing home. The aim was restricted to normal, non-epidemic circumstances, i.e. situations in which the risk of transmission events was presumably within the normal range, and to primary health care settings.

- ✓ To quantify the prevalence of colonization with *S. aureus* and *E. coli* at specific body sites, and to analyse the corresponding degree of AMR (Papers I and II).
- ✓ To determine the molecular epidemiology of *S. aureus*, and the local transmission patterns of its strains (Paper III).
- ✓ To quantify any differences in the molecular epidemiology of *S. aureus* based on the participants' type of accommodation (Paper IV).

SHADES and MIDIO Programmes

Study of Health and Drugs in the Elderly Programme (SHADES)

General Characteristics

We launched "SHADES" (the Study of Health and Drugs in the Elderly) as a non-experimental, longitudinal aggregate (or cohort) study based on elderly nursing home residents. The programme ran between 2008 and 2011 as a joint venture within the three counties of Skåne, Jönköping, and Östergötland, all of which are in southern Sweden.

Recruitment Process

The recruitment process was in part judgemental (i.e. the non-random process of actively seeking persons whose eligibility was established in advance) and in part consecutive (i.e. seeking to include as many persons as possible from within the eligible group). The recruitment was done in two distinct phases. Firstly, the administrators of all nursing homes located within the three counties were contacted by mail. Secondly—provided that the administrators answered favourably—all persons living in the corresponding nursing homes were approached individually for participation (Figure 1). The exclusion criteria were severe illness, palliative care, and language problems. Three experienced study nurses (one nurse in each county) were in charge of the personal contacts with the participants and the sampling procedures done at the nursing homes.

Study Protocol

The *SHADES* study protocol was comprehensive and aimed to multi-purpose scientific application in the field of geriatrics, especially aspects of physical and mental frailty in old age. The participants were scheduled for recurrent assessments and samplings for the duration of the programme. Every six months, the participants were subject of a variety of health assessment scales including the Modified Norton Scale, the Downton Fall Risk Index, and the

Mini Nutritional Assessment Scale, blood sampling, a review of their medical treatment (including antibacterials), and a review of any movements between nursing home and hospital (in conjunction with the corresponding discharge notes) [89 - 91]. Shortly after start-up, the protocol was supplemented with a schedule for microbiological sampling from multiple body sites.

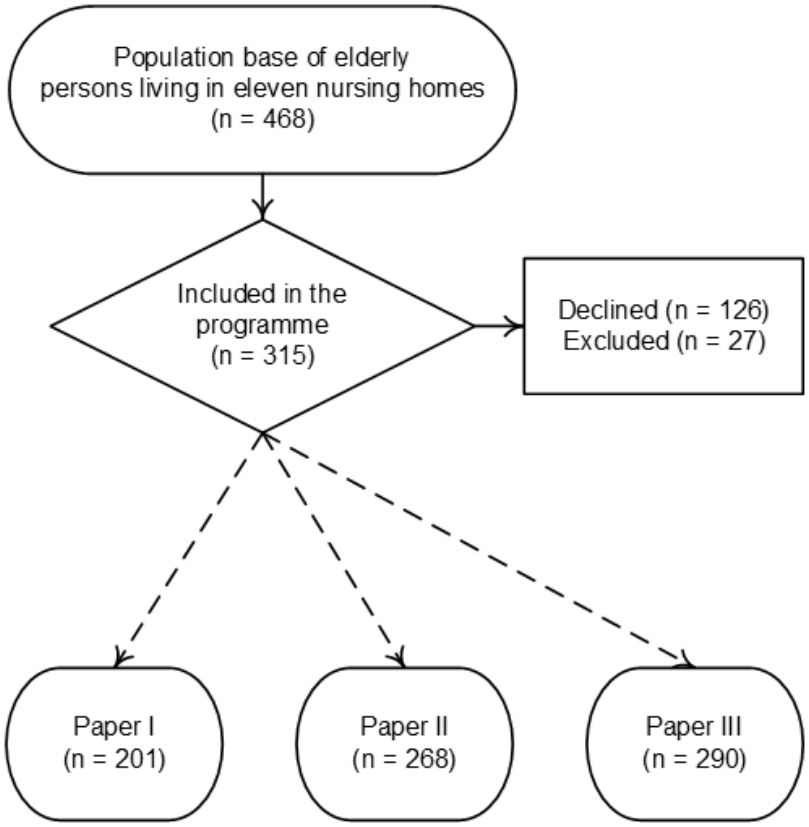

Figure 1. Flow chart of the recruitment process for the *SHADES* programme. The lines in the lower part of the diagram are dashed due to the multiplicity of factors involved in selecting the precise number of participants for each article (see the text for details).

Ethical Considerations

The programme was implemented among elderly people who, with respect to advanced age and associated poor health might be considered disadvantaged in society. However, the programme required access to facilities where people were being cared for in order to fulfil the design and theoretical framework. Therefore, the choice of study population was not considered an undue

exploitation. The procedure for collecting the bacterial specimens was invasive to some degree, given that the swabs had to be introduced into more than one body cavity. However, the slight discomfort associated with swabbing a mucosal surface was deemed acceptable, and the risk of incurring bodily harm was deemed low. The *SHADES* study protocol was approved by the Regional Ethical Review Board at Linköping University (date: October 10, 2007; case number: M150-07). Informed consent was obtained from all participants (or from persons close to them when appropriate).

Participants

The programme was designed as an open aggregate (or open cohort), i.e. new participants were included throughout the duration of the study (as opposed to setting a stop-date after which new participants could not be entered). The open aggregate design resulted in an asymmetric collection of data, in which the number of completed 6-month assessments and samplings was dissimilar among the participants. In addition, there were some inconsistencies in the population sizes reported in the ensuing scientific articles, given that different portions of the data were documented at different points in time, and that microbiological sampling never reached full coverage [92 - 98]. The population base consisted of eleven nursing homes in the three counties of Skåne, Jönköping, and Östergötland. When the programme began in 2008, these nursing homes yielded a maximum of 468 eligible residents. Of these, 315 accepted the request to participate, 126 declined, and another 27 were excluded for various reasons including cooperation difficulties due to deteriorating health. For the ensuing scientific articles devoted to microbiology, data was accessible from 201 participants (Paper I), 268 participants (Paper II), and 290 participants (Paper III), respectively. On the whole, the participants were elderly, Caucasian men and women, and almost all were above the age of 65 years. Paralleling the expected demographics of old age, the majority of the participants were female. All participants lived in nursing homes for the sake of poor general health and disability. Many were physically and mentally frail; many were treated with multiple medications.

Archive Data

The registration of archive data was centralized to the department of research and development in the county of Jönköping. Following the same 6-month cycle as the sampling procedures, the archive data was also reviewed and

upgraded every six months for the duration of the programme. The open aggregate design produced a type of data not unlike repeated-measure data, where information on treatment with antibacterials and hospital visits referred to a specific 6-month window in time. Combining these 6-month windows made it possible to retrace the documentation of prescribed antibacterials as far back as 180 days prior to the first microbiological sampling.

Clinical Observations

The three study nurses (one in each county, see above) were responsible for organizing the clinical observations made at the nursing homes. The clinical observations were concentrated to the recurrent revisions of the health assessment scales.

Microbiological Sampling

The *S. aureus* specimens were collected using a rayon-tipped swab (155C Plastic Rayon White; Copan Italia SpA, Italy) from four body sites, (i) the nasal mucosa, (ii) the pharyngeal mucosa, (iii) the groin, and (iv) skin lesions (if any). The *E. coli* specimens were collected using a rayon-tipped swab (CP125CFE or CP114C; Copan Italia SpA, Italy) from three body sites, (i) the rectal mucosa, (ii) the groin, and (iii) skin lesions (if any). Furthermore, urine samples were assembled using plastic urine collection vials.

Laboratory Work

The laboratory work was a joint venture within the microbiological departments of Jönköping and Halmstad (both located in southern Sweden). The biological specimens—blood samples and microbiological samples alike—were saved in a biobank in Jönköping, where in addition to being analysed, they were frozen and stored for future use. The bacterial specimens were cultivated in accordance with Swedish reference methods [99]. The antimicrobial susceptibility tests were specified using NordicAST minimum inhibitory concentration breakpoints [100]. Further details on procedures and tests can be found in the articles (Papers I - III).

Collection of Antibacterials

For the *S. aureus* specimens, the series of 10 antibacterials (or combination of antibacterials) used in the susceptibility tests included (i) ciprofloxacin, (ii) clindamycin, (iii) erythromycin, (iv) fusidic acid, (v) gentamicin, (vi) linezolid, (vii) rifampicin, (viii) sulphamethoxazole and trimethoprim, (ix) tetracycline, and (x) vancomycin. For the *E. coli* specimens, the series of 12 antibacterials (or combination of antibacterials) used in the susceptibility tests included (i) ampicillin, (ii) cefadroxil, (iii) cefotaxime, (iv) ceftazidime, (v) ciprofloxacin, (vi) mecillinam, (vii) meropenem, (viii) nitrofurantoin, (ix) piperacillin and tazobactam, (x) sulphamethoxazole and trimethoprim, (xi) tobramycin, and (xii) trimethoprim. The 12-item array was not applied consistently, however, given that the initial half of all *E. coli* isolates was analysed according to the 6-item array that was in clinical use at the time. The problem with the sparsely populated collection of antibacterials went unnoticed for some time, leaving only the second half of all isolates analysed according to the complete 12-item array.

Genetic Identification of Bacterial Isolates

The *S. aureus* isolates were identified ("fingerprinted") at the genetic level by sequencing the DNA of the highly variable X region of their *Staphylococcus* protein A gene (*spa* gene). The resulting DNA sequences were then matched to an electronic library holding about 10,000 known types of *spa* genes, thus labelling each matching DNA sequence with a *spa* type designation [101]. Consequently, all *S. aureus* isolates were labelled with a distinctive token (e.g. `t160`) relating to their *spa* type designation. The *spa* types were then used as an indicator of relatedness—if two isolates had the same *spa* type, chances were that they shared a common ancestor. Conversely, if two isolates had different *spa* types, they were more likely unrelated. The precise methodology behind *spa* typing is complicated, but has been described elsewhere [102 - 105].

Microbiological Diversity in Ödeshög Programme (MIDIO)

Additional Value

The *SHADES* Programme established a knowledge base with respect to bacterial colonization, but seemed to provide few insights into bacterial transmission events. At the same time, only a very large, repeated-measure, longitudinal study would be sufficient to identify the base rate, possible high-risk scenarios, and patient-level factors associated with bacterial transmission events. As an alternative, we launched "MIDIO" (the Microbiological Diversity in Ödeshög Programme) as a working model and proxy for bacterial transmission events. The guiding principle was the idea that a crowded physical space maintains its internal colonization pressure by an ongoing process of never-ending transmission events back and forth within the groupings of human hosts [106 - 108]. In pursuing this approach, we theorized that a feedback situation of this type always favours bacterial strains that occur in high numbers, given that strains that are already marginalized might be at a disadvantage in competing for new growth sites. Assuming the working model and proxy to be correct, the model would imply at least two consequences for the human-bacterial ecology, (i) the genetic diversity would decrease with time as marginalized strains gradually perish, and (ii) the remaining diversity would stabilize at a lower level and thus maintain a limited number of highly viable strains of bacteria (i.e. a "mainstream effect" or an "institutional flora" mirroring the institution in question).

General Characteristics

The *MIDIO* Programme was a non-experimental, cross-sectional study based on an elderly population living in either a nursing home or their own place of residence located in the geographical area immediately surrounding the nursing home. The programme was run in late 2014 in the county of Östergötland, which is located in southern Sweden. Specifically, the programme was run in the village of Ödeshög (where there is only one nursing home) with the kind assistance of Ödeshög Health Care Centre.

Recruitment Process

In conformity with the *SHADES* Programme, the recruitment process was in part judgemental and in part consecutive. Two registers formed the base for recruitment, (i) an informal register of all elderly nursing home residents in Ödeshög, and (ii) a register of all persons included in the "Senior Alert" Programme (a nation-wide health care register aimed to reviewing the health status of people above the age of 75 years) within the catchment area of Ödeshög Health Care Centre (Figure 2) [109, 110]. An experienced study nurse approached all eligible persons mentioned in these registers for participation. The exclusion criterion was inability to cooperate due to terminal illness or severe dementia. The same study nurse was also in charge of the sampling procedures done at the nursing home and in the participants' own place of residence.

Study Protocol

By contrast with the extensive *SHADES* study protocol, the limited *MIDIO* study protocol was restricted to aspects believed to be important with respect to commensal bacteria. The *MIDIO* study protocol registered known medical issues, previous treatment with antibacterials, contacts with Ödeshög Health Care Centre and local hospitals in Östergötland, and basic health assessment scales including the disability scale adopted by the Swedish National Study on Ageing and Care Programme [111, 112].

Ethical Considerations

The *SHADES* and *MIDIO* study protocols were similar with respect to ethical considerations, given that there were no major differences regarding how and why the samples were collected. The *MIDIO* study protocol was approved by the Regional Ethical Review Board at Linköping University (date: August 13, 2014; case number: 2014/211-31). Informed consent was obtained from all participants (or from persons close to them when appropriate).

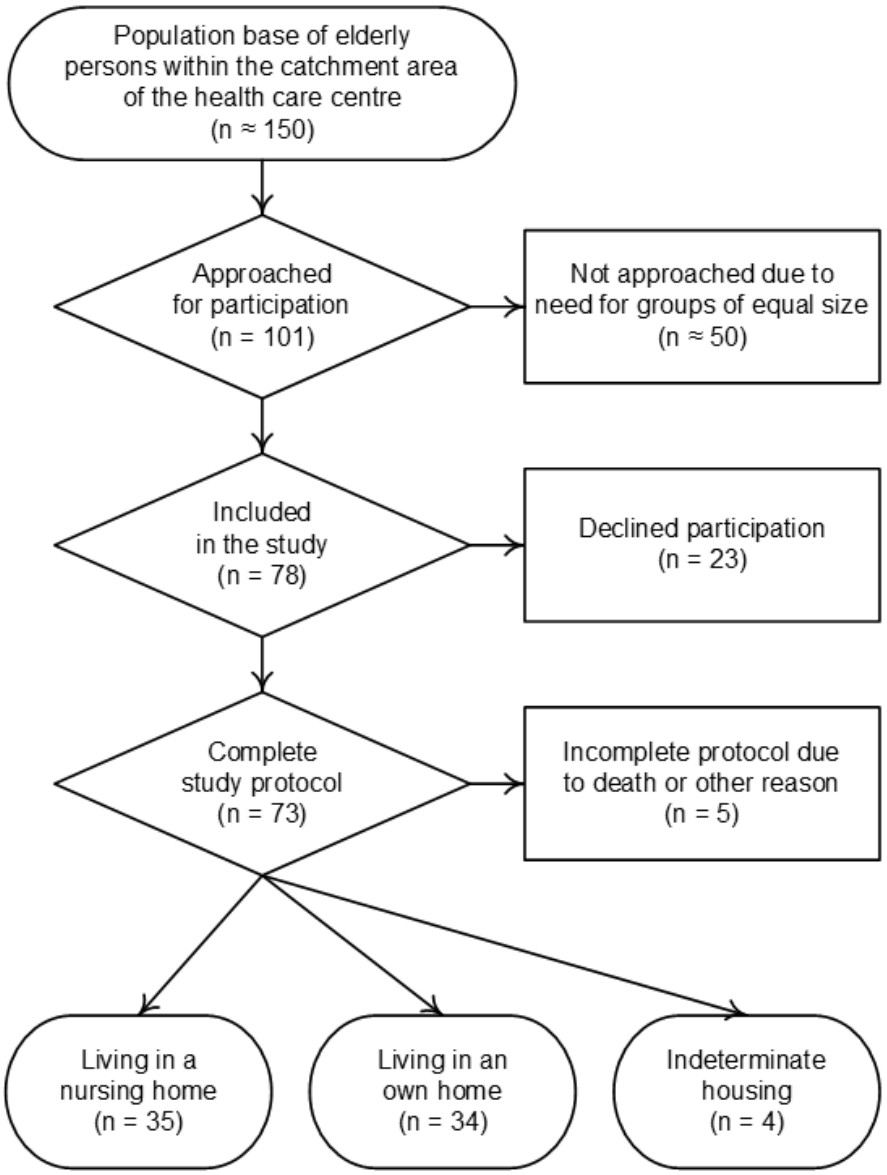

Figure 2. Flow chart of the recruitment process for the *MIDIO* programme.

Participants

The programme was designed as a cross-sectional study. Microbiological sampling was done only one time in the majority of the cases, except for a handful of cases, in which the participant was subjected to sampling more

than once in hopes of gaining insight into the effect of movements between the nursing home and the participant's registered home. This strategy was, however, later abandoned for lack of resources. The population base consisted of a combination of the elderly nursing home residents in Ödeshög and the persons who were registered locally via the *Senior Alert* Programme as described above. All combined, the population base amounted to 150 persons. Of these, about 50 persons were not approached due to a need for groups of equal size. Another 28 were declined participation due to withdrawal of consent (n = 23) or an incomplete study protocol (n = 5). For the ensuing scientific article, data was accessible from 73 participants (Paper IV). On the whole, the participants were elderly, Caucasian men and women. All were above the age of 69 years. Paralleling the expected demographics of old age, the majority of the participants were female. Half of the participants lived in a nursing home for the sake of poor general health and disability; the remaining half lived in their own place of residence and enjoyed relatively good health. In this broad-spectrum patient mix, some participants had little in the way of medical treatment and only rarely visited the health care centre.

Archive Data

Chronic illnesses

Data on chronic illnesses was based on the information stored in each participant's electronic medical record at Östergötland County Council. The registration was restricted to widespread diseases (i.e. uncommon diagnoses were not reviewed), and was done at the most basic level (i.e. the diagnoses were never specified with respect to subtypes or variants, for example the category "diabetes mellitus" was never specified as type 1- or type 2-diabetes). The seven diagnoses registered were (i) cancer of the breast, prostate, or colon, (ii) chronic skin lesions, (iii) dementia, (iv) diabetes mellitus, (v) major fractures of the femur or pelvis, (vi) obstructive lung disease, and (vii) stroke. We were obliged to accept the validity of the diagnoses made by health care professionals who were employed in the public primary health care services, i.e. the diagnoses were neither checked for adherence to international standards nor actively corroborated using other data sources. Medical record entries older than four years at the time of inclusion in the study programme were not reviewed.

Treatment with antibacterials

Data on treatment with antibacterials was based on the information stored in either the participant's electronic medical record at Östergötland County Council or in the on-line overview of the participant's automated medication dispensing system. This service, which is an on-line alternative to collecting medicines in the original packaging from a regular pharmacy, is available in Sweden, but not in all European countries. Swedish patients can apply to a specialized pharmacy for a permanent subscription to medicines, which are then organized and shipped to the patient in envelope-like dose-packages labelled with the date and time the pills inside it are to be taken. The registered antibacterials were classified at all levels of the Anatomic Therapeutic Chemical Classification System (i.e. not only at the most basic level) [113]. Prescriptions older than four years at the time of inclusion in the study programme were not reviewed.

Health care contacts

Due to the extreme number of medical record entries regarding health care contacts and days in hospital (in excess of 5,000 entries in the 4-year period leading up to inclusion in the study programme), manual registration of health care contacts was deemed unrealistic. As an alternative, an excerpt of all the contacts that had been recorded in the electronic booking-system was obtained from the Östergötland County Council database in electronic form. Again, we were obliged to accept the fact that the mixed archive entries referred to a wide range of possible actions taken by health care professionals, i.e. actions not all of which would entail an in-person meeting or other physical route for transferring bacteria.

Clinical Observations

Height and weight

The study nurse collected data on height and weight. If a participant was unable to stand up, his or her height was estimated based on medical record data. All participants were able to use either a portable scale or a special sitting scale.

Impairment

The study nurse assessed the participants' specific impairments with respect to urinary incontinence and mobility impairment (sometimes in collaboration with nursing staff who knew the participant well). The severity of the impairments was rated according to a 4-stage ordinal scale adopted by the Swedish National Study on Ageing and Care Programme in 2014 (the programme has since moved on and is now using different assessment scales) [111]. For the *MIDIO* Programme, the original 4-stage ordinal scale was consolidated to only two stages. The stages "none" and "slight" were designated "no impairment", while the stages "moderate" and "severe" were classified "impairment." Given these designations, urinary incontinence meant that the individual failed to hold urine often, always, or was dependent upon a urinary catheter. Along the same line, mobility impairment meant that the individual was dependent upon walking mobility aids or wheelchairs to make limited indoor movement, or was dependent upon other people to assist with movement.

Skin lesions

The study nurse collected data on skin lesions in the manner deemed most convenient, either by examining the participant herself, collaborating with medical staff, or simply asking the participants who were in good health and could speak for themselves.

Microbiological Sampling

The *S. aureus* specimens were collected using an ESwab™ liquid-based collection and transport system (Copan Diagnostics, Murietta, CA, U.S.A.) from four body sites, (i) the nasal mucosa, (ii) the pharyngeal mucosa, (iii) the groin, and (iv) skin lesions (if any). In addition to the different body sites, samples were also collected from two inanimate sources. These sources were mobility aids for getting in or out of bed (i.e. things the participants were likely to touch every day), and samples of indoor air from the participant's room at the nursing home. Given the low expected rate of positive findings, samples of indoor air were not collected unless at least one *S. aureus* isolate had been recovered earlier from the participant's body.

Laboratory Work

The laboratory work was done at the microbiological department in Jönköping. No specimens other than microbiological specimens were analysed, frozen and stored (thus omitting the need for a full-scale biobank holding traceable human DNA). In conformity with the *SHADES* Programme, the bacterial specimens were handled in accordance with Swedish reference methods and NordicAST minimum inhibitory concentration breakpoints [99, 100]. The methods for *spa* typing were similar to those used in the *SHADES* Programme [103]. Further details on procedures and tests can be found in the article (Paper IV).

Collection of Antibacterials

A series of 16 antibacterials (or combination of antibacterials) was used in the susceptibility tests including (i) amikacin, (ii) cefoxitin, (iii) ciprofloxacin, (iv) clindamycin, (v) erythromycin, (vi) fusidic acid, (vii) gentamicin, (viii) linezolid, (ix) norfloxacin, (x) oxacillin, (xi) penicillin G, (xii) rifampicin, (xiii) sulphamethoxazole and trimethoprim, (xiv) tetracycline, (xv) tobramycin, and (xvi) vancomycin.

Statistical Methods

Paper I

The three main types of statistical analyses were (i) the prevalence of colonization with bacteria at the respective body sites, (ii) the corresponding proportion of AMR isolates, and (iii) univariate odds ratios (OR). The latter reflected the associations within bacterial colonization at the respective body sites and a series of variables divided by age (above or below the median), gender (male or female), and length of stay at the nursing home (above or below the median). The dichotomous variables were all analysed using Pearson's χ^2-test. A p-value of <0.05 was considered statistically significant.

Paper II

For paper II, the analyses were similar to paper I, although the series of variables was expanded to include recent treatment with antibacterials (yes or no), mobility impairment (yes or no), and urinary incontinence (yes or no).

Paper III

For paper III, the statistical analyses were explored even further as compared to papers I and II, given that the growing body of data now included more than one round of microbiological sampling results together with genetic fingerprinting data for the *S. aureus* isolates. The expanded list of analyses included accumulated prevalence of bacterial colonization (aggregating both specific body sites and specific points in time), proportions of specific *spa* types, and a "based upon repeated-patterns" analysis to determine the degree of relatedness within the different strains of *S. aureus* [114, 115].

Paper IV

Univariate Associations

Two types of univariate analysis were utilized. One was univariate OR, the other was a compared means test. Both were used to reflect the associations within the prevalence of bacterial colonization at the respective body sites and a series of background variables, e.g. housing (nursing home or private home), age (years), gender (male or female), treatment with antibacterials (yes or no), hospitalization (days) etc. The occurrence of different *spa* types was presented using the actual numbers (without any central measures attached). Continuous variables were analysed using Student's t-test; dichotomous variables were analysed using Pearson's χ^2-test. A p-value of <0.05 was considered statistically significant.

Multivariate Associations

Three multivariate analyses were done. In each logistic regression model, the six explanatory variables were (i) gender (male or female), (ii) age (years), (iii) body mass index (BMI) (kg/m^2), (iv) diabetes mellitus (yes or no), (v) mobility impairment (yes or no), and (vi) treatment with antibacterials (yes or no). The three outcome variables were (i) colonization with *S. aureus* in the nasal mucosa, (ii) colonization with *S. aureus* in the pharyngeal mucosa, and (iii) colonization with *S. aureus* in the groin. We employed a complex set of principles to guide the choice of variables for the multivariate analyses. These five principles were (i) the outcome variables were never aggregated, given that bacterial colonization at different body sites might not be biologically inter-dependent, (ii) the genetic diversity was never used as an outcome variable, given that the genetic diversity was clearly dependent upon the underlying prevalence of colonization, (iii) only those explanatory variables that divided the dataset in roughly equal parts were used (thus excluding variables that were almost all-positive or all-negative), (iv) only those explanatory variables that were noticeable in the univariate analyses were used (thus excluding variables that were either insignificant or difficult to interpret due to sparse research coverage), and (v) health care contacts were excluded as explanatory variables, given the uncertainty involved in classifying such contacts as a cause or an effect.

Rarefactions

Genetic diversity cannot be quantified using a central measure, given that a mean or median value cannot be applied to nominal data (i.e. attributes that are essentially dissimilar and cannot be combined in a meaningful way) [116, 117]. As an alternative, genetic diversity was illustrated using a basic rarefaction (a rare-faction is a computer-made series of accumulated means showing the overall tendency towards genetic diversity). Following the multivariate analyses described above, a rarefaction can also be divided into subsets by two or more explanatory variables.

The process began with a list of all individuals. Each listed was encoded using the attributes that were to be analysed. In the *MIDIO* Programme, all 73 participants were encoded using the *spa* types found on his or her body and were subsequently divided into subgroups by the same explanatory variables as in the multivariate analyses outlined above. From the list of attributes encoding all participants, a random subset was chosen so that only a certain percentage was represented (e.g. 20 out of 73 encoded individuals). This was followed by counting the number of unique *spa* types lodged within the subset (e.g. 6 unique *spa* types). Due to the role of chance, a single run produces just about any count of unique *spa* types within the population range. Iterating the procedure a number of times, however, will cause the accumulated mean to converge into a number that reflects or mirrors that particular subset (just like the converging tendency towards an accumulated mean of 3.50 when a regular six-sided die is thrown a number of times). In pursuing this approach, a series of random subsets was run and the subset size was increased in a step-wise fashion (e.g. 20, 30, 40…individuals). This yielded a series of accumulated means mirroring each subset size (e.g. 1.23, 2.35, 3.73…unique *spa* types).

The series of accumulated means was plotted in a graph. A plot which is offset to the left is associated with a higher diversity, given a high mean number of unique *spa* types is found in a diverse population even if the subset size is small. Conversely, a plot which is offset to the right is associated with a lower diversity, given a high mean number of unique *spa* types cannot be found in a non-diverse population unless the subset size is large. The rarefactions done in the *MIDIO* Programme were pre-set to be iterated 1,000 times.

Bacterial Colonization in the Nursing Homes

Study of Health and Drugs in the Elderly Programme (SHADES)

Baseline Characteristics

Paper I

A total of 201 participants were included, and 61 (30 %) were males. The participants ranged in age from 61 to 101 years, with a median of 86 years. Their length of stay in the nursing homes ranged from 0 to 14 years with a median of 1.8 years.

Paper II

A total of 268 participants were included, and 72 (27 %) were males. The participants ranged in age from 61 to 102 years, with a median of 85 years. Their length of stay in the nursing homes ranged from 0 to 15 years with a median of 1.4 years.

Paper III

A total of 290 participants were included, and 87 (30 %) were males. The participants ranged in age from 60 to 101 years, with a median of 85 years. Their length of stay in the nursing homes ranged from 0 to 14 years with a median of 1.3 years. Paper III was based partially on the same participants and bacterial isolates as in paper I. This paper, however, was documented at a later time and therefore allowed for a three-fold extension, given that (i) more participants had been included, (ii) more rounds of sampling had been done at the nursing homes, and (iii) genetic fingerprinting information had been added. Extending the programme in this way made the asymmetry of the study protocol more obvious in that some participants had only one complete 6-month round of sampling, while others had up to five complete rounds. For

reasons of clarity, the article was presented in two parts, one being cross-sectional in which only one round of sampling was shown and the other being longitudinal in which one to five rounds of sampling were shown.

Bacterial Colonization

Paper I

A total of 152 *S. aureus* isolates were retrieved from the 201 participants. The prevalence of *S. aureus* colonization in each of the four body sites was (i) 34 % in the nasal mucosa, (ii) 35 % in the pharyngeal mucosa, (iii) 10 % in the groin, and (iv) 54 % in skin lesions. Combining all body sites, the accumulated prevalence of *S. aureus* colonization for the entire group was 50 % (Paper I, Table I).

Paper II

A total of 361 *E. coli* isolates were retrieved from the 268 participants. The prevalence of *E. coli* colonization in each of the four body sites (or body fluids) was (i) 81 % in the rectum, (ii) 48 % in the urine, (iii) 30 % in the groin, and (iv) 13 % in skin lesions. Combining all body sites (and body fluids), the accumulated prevalence of *E. coli* colonization for the entire group was 78 % (Paper II, Table I).

Paper III

In the cross-sectional part of the programme, a total of 185 *S. aureus* isolates were retrieved from the 290 participants. The prevalence of *S. aureus* colonization in each of the four body sites was (i) 31 % in the nasal mucosa, (ii) 34 % in the pharyngeal mucosa, (iii) 10 % in the groin, and (iv) 41 % in skin lesions. Combining all body sites (minus skin lesions), the accumulated prevalence of *S. aureus* colonization for the entire group was 48 %. In the longitudinal part of the programme, a total of 466 *S. aureus* isolates were retrieved from the 290 participants (of which 72 % had been sampled more than one time). Subjecting a majority of the participants to repeated sampling in this way increased the aggregated likelihood of recovering a *S. aureus* isolate. The accumulated prevalence for the nasal mucosa rose from 31 % (one sampling) to 60 % (aggregating between one and four samplings). For the pharyngeal mucosa, the corresponding rise went from 34 % to 62 %. A total of 144 (50 %) participants underwent sampling three times or more, but only 44

(15 %) participants had a complete sampling protocol for all body sites three times or more. Among the 144 participants who underwent sampling three times or more, 44 % demonstrated colonization with *S. aureus* in the nasal mucosa on at least one occasion, and 20 % were colonized in the nasal mucosa on all occasions. Among the 44 participants who had a complete sampling protocol three times or more, 70 % demonstrated colonization with *S. aureus* in any body site on at least one occasion, and 25 % were colonized in any body site on all occasions. In other words, a *S. aureus* isolate could be recovered somewhere on their body on all occasions (Paper III, Table 2).

Antimicrobial Susceptibility

Paper I

Of the 152 *S. aureus* isolates, 24 (16 %) came back resistant to any of the 10 tested antibacterials. Specifically, 21 isolates were resistant to ciprofloxacin, one isolate to fusidic acid, one isolate to tetracycline, and one isolate to sulphamethoxazole and trimethoprim. There was no MRSA in the dataset. The 24 resistant isolates were retrieved from 17 participants, corresponding to an AMR prevalence of 18 % in the subset of colonized individuals and 8.5 % in the entire group (colonized and non-colonized groups combined) (Paper I, Table II).

Paper II

Of the 361 *E. coli* isolates, 87 (24 %) came back resistant to any of the 6 or 12 tested antibacterials. Combining the two sets of tested antibacterials, the majority of AMR found in the population was associated with either trimethoprim or ciprofloxacin. There were only two ESBL isolates in the dataset, and both were recovered from the same individual. The 87 resistant isolates were retrieved from 49 participants, corresponding to an AMR prevalence of 23 % in the subset of colonized individuals and 18 % in the entire group (Paper II, Tables II and III).

Paper III

Of the 466 *S. aureus* isolates, 338 (73 %) were tested for antimicrobial susceptibility. Of the 338 tested isolates, 44 (13 %) came back resistant to any of the 10 tested antibacterials. There was no MRSA in the dataset. The 44 resistant isolates were retrieved from 26 participants, corresponding to an AMR

prevalence of 18 % in the subset of colonized individuals and 9.0 % in the entire group.

Genetic Identification of Bacterial Isolates

Paper III

In the cross-sectional part of the programme, the 185 (100 %) *S. aureus* isolates were subject of *spa* typing. A total of 73 unique *spa* types were identified, of which the two most common were `t002` covering 8.3 % of all isolates and `t160` covering 5.8 % of all isolates. The remaining 71 unique *spa* types accounted each for covering 5 % or less of all *S. aureus* isolates (Paper III, Table 1). At the patient level, 13 of the 73 unique *spa* types were represented in three or more participants, while the remaining 60 unique *spa* types were represented in two participants or fewer. In the longitudinal part of the programme, the research coverage came back too sparse to reliably demonstrate any expansion or contraction of the small subsets of *spa* types. A "based upon repeated-patterns" cluster analysis revealed 10 clusters of interrelated *spa* types and 14 individual clusters (or singletons, i.e. *spa* types for which no genetic relationships could be demonstrated). Of the 10 interrelated clusters, *spa*-CC `015` was the most comprehensive, covering 18 % of all unique *spa* types.

Univariate Associations

Paper I

Men demonstrated colonization with *S. aureus* in the nasal mucosa to a greater extent than women (OR 1.9, 95 % CI 1.0 to 3.7). Those who had stayed in the nursing home longer than the median demonstrated colonization with *S. aureus* in the pharyngeal mucosa to a greater extent compared to those who had stayed in the nursing home shorter than the median (OR 2.0, 95 % CI 1.0 to 4.0). There were no other statistically sound univariate associations with respect to age or recent hospitalizations. Furthermore, there were no statistically sound univariate associations with respect to colonization with AMR isolates and any of the variables listed above.

Paper II

Men demonstrated colonization with *E. coli* in the urine and groin to a lesser extent than women (OR 0.13, 95 % CI 0.05 to 0.31 and OR 0.47, 95 % CI 0.23 to 0.95, respectively). Men also demonstrated colonization with AMR *E. coli* in the rectum to a lesser extent than women (OR 0.33, 95 % CI 0.12 to 0.90). Those aged above the median demonstrated colonization with *E. coli* in the urine to a greater extent than those below the median (OR 3.1, 95 % CI 1.7 to 5.8). Those suffering from severe urinary incontinence (stage 4 of the disability scale adopted by the Swedish National Study on Ageing and Care Programme) demonstrated colonization with *E. coli* in the groin to a greater extent than those with less severe or no urinary problems (OR 4.9, 95 % CI 2.4 to 9.8). Those treated with antibacterials during the 180-day period preceding sampling demonstrated colonization with AMR *E. coli* in the rectum to a greater extent than those not treated (OR 3.3, 95 % CI 1.5 to 7.0). There were no other statistically sound univariate associations with respect to length of stay at the nursing home or to mobility impairment (Paper II, Table II).

Paper III

Those participants who carried more than one *spa* type on their body were more likely to carry a *S. aureus* isolate that was resistant to any antibacterial ($p = 0.04$). Apart from this, there were very few univariate associations in the dataset that was not documented previously in paper I.

Main Findings

Paper I

Active surveillance of AMR done on 201 Swedish elderly nursing home residents showed that a *S. aureus* isolate could be retrieved from 1 in every 2 individuals, and that the overall prevalence of AMR was favourable when compared to the situation in many other countries. Resistance to fluoroquinolones was a cause for concern even in a setting with a low prevalence of AMR.

Paper II

Active surveillance of AMR done on 268 Swedish elderly nursing home residents showed that an *E. coli* isolate could be retrieved from 4 in every 5 individuals, and that the overall prevalence of AMR was favourable when

compared to the situation in many other countries. Resistance to trimethoprim and fluoroquinolones were the most important AMR concerns found, thereby lending credence to the idea that fluoroquinolones given orally should not be used to treat lower urinary tract infections.

Paper III

Active surveillance of AMR done on 290 Swedish elderly nursing home residents showed that a *S. aureus* isolate could be retrieved from 1 in every 2 individuals given a single round of sampling. However, in an aggregate sampling in several rounds, this figure might reach 7 in every 10 individuals. The overall prevalence of AMR was favourable when compared to the situation in many other countries. The distribution of *spa* types among the participants was diverse and provided only limited evidence of clonal expansion or contraction. Certain *spa* types (e.g. `t160`) might have been more common on the whole, but there were few significant univariate associations within specific *spa* types and patient-level factors (e.g. gender and age). The elevated risk of AMR in participants colonized with more than one *spa* type raised a concern for "hidden" resistance (i.e. a risk of reporting an incorrect degree of susceptibility following a failure to separate AMR strains from susceptible strains in routine clinical samples).

Microbiological Diversity in Ödeshög Programme (MIDIO)

Baseline Characteristics

A total of 73 participants were included, and 25 (34 %) were males. The participants ranged in age from 69.9 to 98.5 years, with a median of 83.8 years. The participants were divided into three groups based on the type of accommodation. The number of participants in each group were 35 (48 %) in the nursing home group, 34 (47 %) in the unassisted living group, and 4 (5 %) in the indeterminate group (due to recent movements between nursing home and own place of residence). Of the elderly nursing home residents, 8 (23 %) had lived there longer than the analytical ceiling of about four years. Within the nursing home group, the median time for living there was 1.3 years. The prevalence of the seven widespread diseases was (i) cancer of the breast, prostate, or colon 14 %, (ii) chronic skin lesions 11 %, (iii) dementia 16 %,

(iv) diabetes mellitus 38 %, (v) major fractures of the femur or pelvis 16 %, (vi) obstructive lung disease 8 %, and (vii) stroke 18 %. A total of 859 days of hospitalization and 5,055 primary care contacts were registered in the 36-month period preceding inclusion in the study programme. A total of 1,940 days of treatment with antibacterials were registered in the 36-month period preceding inclusion in the study programme. The patient-level risk for registering treatment with each of the Anatomic Therapeutic Chemical Classification System subgroups was J01A (tetracyclines) 14 %, J01C (beta-lactam antibacterials, penicillins) 58 %, J01D (other beta-lactam antibacterials) 36 %, J01E (sulfonamides and trimethoprim) 4 %, J01F (macrolides, lincosamides and streptogramins) 8 %, J01G (aminoglycoside antibacterials) 1 %, J01M (quinolone antibacterials) 16 %, and J01X (other antibacterials) 15 % (Paper IV, Table 1).

Bacterial Colonization

A total of 69 *S. aureus* isolates were retrieved from the 73 participants. The prevalence of *S. aureus* colonization in each of the four body sites was (i) 34 % in the nasal mucosa, (ii) 26 % in the pharyngeal mucosa, (iii) 21 % in the groin, and (iv) 33 % in skin lesions. Combining all body sites, the accumulated prevalence for the entire group was 47 %. In addition, a *S. aureus* isolate was recovered from 5 out of 23 (22 %) tested mobility aids. However, these isolates were excluded from further analyses due to the low expected statistical impact. Furthermore, the sampling of indoor air resulted in only one *S. aureus* isolate (which was also excluded for the same reason).

Antimicrobial Susceptibility

All 69 (100 %) *S. aureus* isolates came back resistant to penicillin G. Apart from this, only one isolate (1.4 %) came back resistant to four of the remaining 15 tested antibacterials. These four antibacterials were ciprofloxacin, clindamycin, erythromycin, and norfloxacillin. There was no MRSA in the dataset. The resistant *S. aureus* isolate was retrieved from one participant, corresponding to an AMR prevalence of 1.4 % in the entire group (colonized and non-colonized groups combined).

Genetic Identification of Bacterial Isolates

All 69 (100 %) *S. aureus* isolates were subject of *spa* typing. A total of 29 unique *spa* types were identified, of which the four most common were `t5593` covering 10 % of all isolates, `t085` covering 8.7 % of all isolates, `t160` covering 8.7 % of all isolates, and `t14905` covering 8.7 % of all isolates. The remaining 25 unique *spa* types accounted each for covering 6 % or less of all *S. aureus* isolates. In the nursing home group, the most common *spa* types were `t160` and `t14905`. In the unassisted living group, the most common *spa* types were `t252`, `t342`, and `t5593` (Paper IV, Table 2).

Univariate Associations

The mean BMI was higher among participants diagnosed with diabetes mellitus ($p = 0.004$). The mean BMI was lower among participants diagnosed with chronic skin lesions ($p = 0.015$). Those colonized with *S. aureus* in the nasal mucosa were diagnosed with diabetes mellitus to a greater extent ($p = 0.006$), were treated with more antibacterials ($p = 0.048$), and registered more primary health care contacts ($p = 0.012$ or $p = 0.029$ depending on the frequency of visits). Those colonized with *S. aureus* in the pharyngeal mucosa were treated with antibacterials to a greater extent ($p = 0.028$ or $p = 0.001$ depending on the preparation and frequency of treatment), and registered more primary health care contacts ($p = 0.048$ or $p = 0.021$ depending on the frequency of visits). Those colonized with *S. aureus* in the groin were diagnosed with diabetes mellitus to a greater extent ($p = 0.011$) and registered a more severe mobility impairment ($p = 0.024$). No statistically sound univariate associations were registered within any AMR category at any body site.

Multivariate Associations

In the three logistic regressions, the only significant result was that diabetes mellitus was associated with a higher degree of colonization with *S. aureus* in the nasal mucosa (OR 4.44, 95 % CI 1.34 to 14.66) (Paper IV, Table 3).

Rarefactions

Two rarefactions were run separately. The first rarefaction compared the genetic diversity within the nursing home group and the unassisted living group; the second rarefaction compared the genetic diversity within the seven

explanatory variables used in the multivariate analyses (see above). These seven variables were diabetes mellitus (yes or no), antibacterial treatment in a high range intensity within the 36-month period preceding inclusion in the study programme (yes or no), mobility impairment (yes or no), age (above or below the median), BMI (above or below the median), gender (male or female), and housing (nursing home or private home). In the first rarefaction model, the tendency towards genetic diversity was almost identical in the nursing home group and the unassisted living group, given that the two plots overlapped. Our interpretation was that the presence of a biological process that acts as a limiter or equalizer in the nursing home group was unlikely (Paper IV, Figure 2a). In the second rarefaction model, the tendency towards genetic diversity was higher among those diagnosed with diabetes mellitus, given that the plot was offset to the left. There was a similar, although weaker, tendency towards a higher diversity among those treated with antibacterials and those with mobility impairment. Our interpretation was that the presence of a biological mechanism that acts as a booster is likely and that such a mechanism might be associated with the metabolic changes occurring as a result of diabetes mellitus (Paper IV, Figure 2b).

Main Findings

Active surveillance of AMR in a group of 73 elderly Swedish participants, half of which were nursing home residents, and the remaining half lived in their own place of residence, showed that a *S. aureus* isolate could be retrieved from 1 in every 2 individuals, and that the overall prevalence of AMR was favourable when compared to the situation in many other countries. The distribution of *spa* types among the participants was diverse and provided no evidence of clonal expansion or contraction. Furthermore, the *spa* types inside and outside the nursing home were—although different with respect to overall composition—equally diverse, rendering a depletion of the genetic diversity in the nursing home in the form of a "mainstream effect" unlikely. Diabetes mellitus came out as the most powerful driver of genetic diversity in this population.

General Discussion

Active surveillance of AMR in different groups of elderly Swedish people, living in or around a nursing home, showed that a *S. aureus* isolate could be retrieved from 1 in every 2 individuals given a single round of sampling. However, in an aggregate sampling in several rounds, this figure might reach 7 in every 10 individuals. An *E. coli* isolate could be retrieved from 4 in every 5 individuals, and the overall prevalence of AMR was favourable when compared to the situation in many other countries. Further, the distribution of *S. aureus spa* types among the participants was diverse and provided only limited evidence of clonal expansion or contraction. Certain *spa* types (e.g. `t160`) were more common on the whole, but there was little evidence of any associations within specific *spa* types and patient-level factors (e.g. gender or age). The elevated risk of AMR in participants colonized with more than one *S. aureus spa* type raised a concern for "hidden" resistance (i.e. a risk of reporting an incorrect degree of susceptibility following a failure to separate AMR strains from susceptible strains in routine clinical samples). Comparing the *spa* types found inside and outside a nursing home, the *spa* types were different in overall composition, yet equally diverse. Consequently, any depletion of the genetic diversity in the nursing home in the form of a "mainstream effect" seemed unlikely. Diabetes mellitus was one of very few patient-level factors to show an association with the degree of genetic diversity in colonizing strains of *S. aureus*.

The prevalence of *S. aureus* colonizing strains was somewhat higher than that registered in two recent European studies [118, 119]. This was possibly due to the advanced mean age of the participants in our study. However, the prevalence of *S. aureus* has been known to vary dramatically within different investigations, thus making far-reaching conclusions difficult [120, 121]. The prevalence of *E. coli* colonizing strains is also difficult to comment on, given that the discrepancy between our result and a pro forma 100 % colonization might in part be due to methodological problems. The low prevalence of multi-drug-resistant organisms is an expected finding, bearing in mind the low prevalence of MRSA and ESBL in the Scandinavian countries. In Sweden, the estimated incidence of MRSA in 2015 was 39 per 100,000 inhabitants, while the parallel incidence of ESBL was 99 per 100,000 inhabitants [122]. Similarly, the tendency towards a high degree of genetic diversity in

colonizing strains of *S. aureus* has also been seen in other studies [123 - 126]. To the best of our knowledge, our study of ecological niches that are closely related, yet physically separated, does not have many predecessors in the field of infectious epidemiology. We also believe that our finding of a similar genetic diversity in colonizing strains of bacteria collected inside and outside a nursing home might be unique. Co-morbidities have been investigated in a recent review article, in which congestive heart failure, diabetes mellitus, pulmonary disease, immuno-suppression, and renal failure were associated with colonization with MRSA [127]. However, another comprehensive review of the subject failed to identify any tenable associations within colonization with MRSA and patient-level factors [128].

Although a poignant combination, the duality of group-living and physical and mental frailty among Swedish elderly nursing home residents seems to have little effect on the human-bacterial ecology. We have found few signs that nursing homes actually act as reservoirs for multi-drug-resistant organisms in the sense that nursing homes somehow maintain a higher-than-average prevalence of such organisms. In our studies, the bacterial colonization of the elderly nursing home residents only seemed to mirror the bacterial colonization of those living in the geographical area surrounding the nursing home. Extending the argument, this would be an indication that the risk of transmission events within the nursing home population and unassisted living population is in fact similar, and that the association within transmission events and possible "mainstream effects" is unclear. The possible role of diabetes mellitus is interesting, given that diabetes mellitus has been identified as a risk factor for colonization with MRSA in earlier studies (see above), and that diabetes mellitus has been associated with both an elevated risk of infection and a higher-than-average consumption of antibacterials [129 - 133]. Consequently, the capacity of the diabetic patient to retain bacteria on his or her mucosae might constitute a "missing link" between diabetes mellitus and infections [134 - 136].

Methodological Considerations

We believe that active surveillance of AMR is necessary to quantify the volume and dynamics of AMR with a high degree of accuracy. The drawbacks appear to be increasing, however, given that the high cost is followed by a low occurrence of positive findings. This in turn has made sparse research

coverage a salient issue. We believe the strength of our programmes lay in the high degree of sampling consistency, given that the bacterial samples were taken by a limited number of study nurses, taken within a concentrated period of time, and analysed at a limited number of laboratories. Contextual adequacy was also significant since the programmes were run within the context of primary health care. We believe the main limitations were due to small sample sizes and a non-random recruitment process. Possible bias associated with analysing participants at an advanced age might however be found in that the participants were "survivors" and might have represented a subset of individuals that was more resistant to biological degeneration than the actual average. In other words, the diseases were not "killers", and the severity of the participants' diseases might have been within the lower range when compared to the actual average of disease severity. The possible reporting bias such as medication and health care contacts might also have played a role. We are also aware of the possible recruitment bias in connection with having public institutions volunteer to participate, possibly favouring institutions that might be better managed than others.

Using indicator bacteria and a known reservoir for such bacteria as working models and proxies for a more general human-bacterial ecology requires a sense of trust in the model—especially if our findings are to be applied to different contexts or different bacterial species. However, we believe that we have gone to some length in selecting the most relevant infectious agents with respect to nosocomial infections.

Our study utilized hospital quality hygiene in the nursing homes. A considerable proportion of the participants lived in a single room with a private bathroom. Hospital quality hygiene might have contributed to the scarcity of statistical associations within bacterial colonization and patient-level factors and to the preservation of genetic diversity among the strains of colonizing bacteria.

There were at least two influential, internal dependencies in our datasets; namely the genetic diversity of the bacterial strains, which was clearly dependent upon the underlying prevalence of bacteria, and the high prevalence of diabetes mellitus that appeared to be dependent upon the advanced mean age of the populations.

Investigating the prevalence of microbiological material on human beings using a cultivation-dependent methodology might imply some precision problems due to the static properties of the variables. As a rule, the variables in our studies were non-continuous (preventing the use of fine distinctions like Student's t-test) and indivisible (preventing the practice of splitting, combining, or otherwise "tweaking" groups to enhance inter-group comparability). It was also found that the variables were sparse, i.e. holding a high proportion of non-values such as "null" or "undefined" that could not be used for computation. The variables were also unstable in that a grouping made today cannot be relied on tomorrow due to the influence of chance when living bacteria are collected from living mucosal surfaces. In addition, the problem with static variables tended to multiply, given that the need to differentiate between every growth-site, every antibacterial compound etc., left the researchers with confusingly high numbers of static variables. In an attempt to avoid further confusion and data losses, we decided to use a basic rarefaction model, i.e. a model that was crude enough to accept all available data as input regardless of sparsity or other less-than-ideal properties.

Relying on colonization with commensal bacteria to discriminate between groups of human hosts might raise questions with respect to validity. The reason for this stems from the fact that the underlying prevalence of bacteria is dependent upon the self-regulating and self-limiting capacity of the bacterial species in question. In the case of *E. coli*, where the prevalence exceeds 80 %, almost any comparison (and associated statistical inference test) can be rendered useless if the remaining, non-colonized individuals are too few to form a distinctive grouping. Furthermore, the intensity of antibacterial treatment might also have a questionable ability to discriminate between groups, given that the day counts of antibacterial treatment could well be time-dependent. In the *MIDIO* Programme, we found that if a relatively short space of time was included in the comparison (e.g. the 12-month period preceding inclusion in the study programme, which is a common practice in many studies), the discrimination between the different treatment groups seemed valid. In other words, there was an acceptable balance between the percentage that received antibacterial treatment, and the percentage that did not. However, when we extended the time-frame, e.g. from the 12-month period to the 36-month period preceding inclusion in the study programme, the difference between the groups seemed to vanish. Given the irregular use of antibacterials, the high-intensity consumers seemed to blend with the low-

intensity consumers as the majority of the participants received at least some form of treatment within the longer space of time.

Implications for Clinical Practice

Active surveillance of AMR might provide useful feedback for hospital hygiene interventions or even identify transmission events as they occur. Such information might provide the tools for implementing hospital quality hygiene strategies more efficiently.

Active surveillance of AMR might facilitate antibiotic stewardship in as much as changes in AMR can be monitored as they occur in the target populations [137 - 140]. Systematic surveillance involving multiple body sites might also reveal important or rare occurrences of AMR that would otherwise go undetected in routine sampling.

In our data, only a few tenable associations have emerged at the patient-level that might form the basis for directing or restricting screening procedures to at-risk populations. However, the combination of diabetes mellitus and old age might provide fresh suggestions for future investigations in the field of host-pathogen interaction.

Future Research

We have reason to believe that colonization with commensal bacteria is a complicated biological process. A simplistic view of colonization might not be entirely productive, e.g. stating that there is “no smoke without fire” (i.e. the physical proximity of a contamination source will inevitably lead to contamination) or a predictable “colonization pressure” (i.e. the physical proximity of different colonizing strains will lead to interaction between them not unlike the electro-chemical balance that happens between ions in a liquid solution). We suspect that bacterial colonization is quite stable and long-standing and that it is more sensitive to interaction within microbes and hosts than to the possible physical proximity of other microbes and other hosts. Our suspicions—together with the statistical problems outlined above—have led us to believe that the study of commensals might benefit from being regarded as a distinctive sub-discipline in the field of infectious medicine and as a research area in its own right. Consequently, the study of commensals should

be in charge of its own terminology and performance indicators. Instead of relying on traditional views and loosely formulated conventions for quantifying hard-to-handle variables such as colonization density and treatment intensity, there should be benchmarks that are tested and proven. In a field where the research coverage is sparse and the statistical associations are few, benchmarks—crude or over-simplified—are still necessary to demonstrate trends as they happen. The research process might also benefit from shifting the focus from quantifying statistical associations within the microbes and hosts to somehow quantifying the strength of the bond between microbes and hosts, at least in the biochemical sense of the word.

We believe that the *SHADES* and *MIDIO* Programmes have illustrated that primary health care can be suitable for studies in pragmatic epidemiology or "what takes place in the trenches." Lacking the enticing aura of technical sophistication—in the words of Ian R. McWhinney, lacking a "mysterious apparatus"—primary health care is obliged to rely heavily on contextual robustness as its main route for achieving scientific value [141]. Consequently, studies done in primary health care might have a dual significance in the field of infectious medicine, since infections are not only typical and characteristic of a particular geographical area, but they are also spread within the immediate geographical area. In addition, primary health care might be suited for studies in general geriatrics, given that elderly patients tend to visit the health care centre often. This results in increased documentation for these patients. Due to ongoing changes in population dynamics, the proportion of elderly patients is expected to grow steadily in the decades to come [142 - 145].

In traditional infectious epidemiology, a single encounter is enough. It is enough for transmitting influenza and many other droplet-borne infectious diseases. In the realm of commensal bacteria, however, in which continuous contact within microbes and hosts is unavoidable, there might be new aspects to take into account, such as how infectious epidemiology is being approached by scientists and physicians. One important question is how the emerging cultivation-independent methods for diagnosing infections might affect clinical routines in the future and if such methods could be better suited in creating valid distinctions between groups, thereby facilitating the identification of populations at risk for bacterial colonization and nosocomial infection [146 - 148].

Conclusions

The general conclusions drawn in active surveillance of AMR to groups of elderly people in and around nursing homes demonstrated that the number of associations within bacterial colonization and patient-level factors was low, and that variables were static and difficult to handle statistically. Most importantly, it was found that hospital quality hygiene probably contributed to better results with respect to AMR, but also to the statistical difficulties outlined above.

- ✓ The prevalence of colonization with *S. aureus* and *E. coli* was somewhat higher than expected, but the degree of AMR was very low (Papers I and II).
- ✓ The genetic diversity of *S. aureus* was generally high and there were few signs of clonal expansion or contraction (Paper III).
- ✓ The genetic diversity of *S. aureus* was similar within the different types of accommodation, and there were few signs of a depletion of the genetic diversity due to effects of group-living (or "mainstream effect"). Diabetes mellitus emerged as the only patient-level factor associated with a higher degree of genetic diversity among the *S. aureus* isolates (Paper IV).

Populärvetenskaplig sammanfattning

De bakterier som finns runt omkring oss, och även på våra kroppar, fortsätter att bli allt mer motståndskraftiga mot antibiotika. Problemet beror på många olika faktorer, både hos bakterierna själva, och hur vi som människor lever och formar vårt samhälle. Det är mycket svårt att göra något åt problemet, eftersom det inte räcker med att ta fram nya antibiotika – hittills har det alltid dykt upp nya mekanismer för motståndskraft hos bakterierna i samma takt som nya antibiotika har börjat användas. Världshälsoorganisationen (WHO) anser att flera olika strategier behöver användas samtidigt för att bemöta problemet, till exempel utbildning om hur man använder antibiotika på bästa sätt. Det verkar dock som att den allra viktigaste strategin är att övervaka och begränsa spridningen av höggradigt motståndskraftiga bakterier.

Inom vårt projekt har vi tagit prov på bakterier från till exempel näsa och mun hos människor som bor på äldreboenden i syfte att kartlägga sådana boenden vad gäller förekomst och spridning av motståndskraftiga bakterier. Vi har funnit att motståndskraften generellt sett är låg och att svenska äldreboenden inte verkar utgöra någon reservoar för bakterier med en högre eller hittills okänd motståndskraft. Vi bedömer att detta är en effekt av att förekomsten av höggradigt motståndskraftiga bakterier varit låg fram tills nu i de skandinaviska länderna, och att de boenden som undersökts haft välfungerande rutiner för sjukhushygien. Vi har också funnit att risken för spridning av bakterier mellan personer som bor på äldreboenden verkar vara låg, men att diabetes kan vara en riskfaktor för att bära mer bakterier på kroppen.

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