

Diagnostic aspects of urinary tract infections among elderly residents of nursing homes



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ABSTRACT

Background: Up to half the residents of nursing homes for the elderly have asymptomatic bacteriuria (ABU), which should not be treated with antibiotics. Thus, it is difficult to know if new symptoms in residents with bacteriuria are caused by urinary tract infection (UTI), or if bacteriuria only represents an ABU. This is especially difficult in the presence of non-urinary tract specific symptoms. The diagnostic uncertainty is likely to generate significant over-treatment with UTI antibiotics.

Aim: The general aim was to clarify the association between symptoms, bacteriuria, dipstick urinalysis and urine Interleukin-6 (IL-6) among nursing home residents to improve the diagnostic procedure of a suspected lower UTI.

Methods: In 2003 a study protocol including newly onset symptoms was completed, and single voided urine specimens collected for dipstick urinalysis and cultures from 651 residents of 32 participating Swedish nursing homes for the elderly. This data was used for a study of dipstick urinalysis (Paper I) and for a study of nonspecific symptoms and bacteriuria (Paper II). In 2012, similar data was collected for 421 elderly residents of 22 nursing homes, which also included an analysis of IL-6 in urine and urine specimens from another 59 residents with urinary catheters. The association between bacteriuria, IL-6 in urine, dipstick urinalysis and newly onset symptoms was analysed (Paper III). Antimicrobial resistance rates were described among residents of nursing homes in 2012 and compared with those from 2003 (Paper IV).

Results: Paper I: The negative predictive value for predicting absence of bacteriuria was 88 (84-92)% when dipstick urinalysis for nitrite and leukocyte esterase were simultaneously negative. A positive dipstick or any combination thereof could not sufficiently predict bacteriuria.

Papers II-III: New or increased nonspecific symptoms were common among elderly residents of nursing homes. Residents without nonspecific symptoms had positive urine cultures as often as those with nonspecific symptoms with a duration of up to one month.

Paper III: Residents with positive urine cultures had higher concentrations of IL-6 in the urine. However, among residents with positive urine cultures there were no differences in IL-6 concentrations or dipstick findings between those with or without nonspecific symptoms.

Paper IV: The average rates of antimicrobial resistance were low and did not increase between 2003 and 2012 in *Escherichia coli* (*E. coli*) urinary isolates among Swedish nursing home residents. Any antibiotic treatment during the last month and hospitalization during the last six months predicted higher resistance rates among *E. coli*.

Conclusions: Nonspecific symptoms among elderly residents of nursing homes are unlikely to be caused by bacteria in the urine. Therefore, dipstick urinalysis, IL-6 in the urine and urine cultures are of little or no value in clarifying the aetiology of nonspecific symptoms. If there is a reason for testing for bacteriuria, dipstick urinalysis for nitrite and leukocyte esterase can rule out but cannot reliably rule in bacteriuria. Antimicrobial resistance in urinary pathogens among Swedish nursing home residents remained low. It is important to use antibiotics rationally to preserve the effectiveness of antibiotics.

Keywords: Bacteriuria, Nursing Homes, Homes for the Aged, Urinary Tract Infections, Dipstick Urinalysis, Diagnostic Tests, Predictive Value of Tests, Family Practice, Interleukin-6, *Escherichia coli*, Anti-Bacterial Agents, Drug Resistance; Bacterial.

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SAMMANFATTNING PÅ SVENSKA

Avhandlingen avser forskning kring bakterier i urinen, diagnostiska metoder och diffusa symtom bland vårdtagare på äldreboenden. Syftet var att svara på följande frågeställningar: Hur väl kan urinstickor påvisa eller utesluta förekomst av bakterier i urinen bland patienter på äldreboenden? Orsakas diffusa symtom hos patienter på äldreboenden av bakterier i urinen? Kan interleukin-6 (IL-6) vara till hjälp vid diagnostik av misstänkta urinvägsinfektioner på äldreboenden? Hur vanligt förekommande är antibiotika-resistenta urinvägsbakterier bland vårdtagare på äldreboenden?

Första och andra delarbetet baserades på en datainsamling 2003 från 651 vårdtagare på svenska äldreboenden. Följande nytillkomna symtom registrerades; trötthet, oro, förvirring, agitation/ilska, en beskrivning av att inte vara sig lik, täta urinträngningar, sveda vid vattenkastning och feber. Kastade urinprov analyserades med urinodling samt urinstickor med avseende på nitrit och leukocytesteras. Första delarbetet visade att man med hög sannolikhet kan utesluta bakterier i urinen om båda urinstickorna är negativa. Däremot kunde positiva urinstickor inte säkerställa förekomst av bakterier i urinen på grund av låga positiva prediktiva värden. Manuell och maskinell avläsning av urinstickorna hade en acceptabel överensstämmelse.

Andra delarbetet visade att urinodlingar inte bidrar med relevant information vid undersökning av patienter på äldreboenden med trötthet, oro, förvirring, agitation/ilska, sveda vid vattenkastning eller täta urinträngningar.

Tredje och fjärde delarbetet baserades på en datainsamling 2012 där symtom registrerades och urinprov togs från 480 vårdtagare på svenska äldreboenden. Följande nytillkomna diffusa/ospecifika symtom studeras; trötthet, oro, förvirring, agitation/ilska, nedsatt aptit, falltendens, en beskrivning av att på annat sätt inte vara sig lik samt följande urinvägsspecifika symtom; täta urinträngningar, sveda vid vattenkastning och frekventa vattenkastningar. Urinproven analyserades med urinstickor, urinodling inklusive resistensbestämning samt genom att mäta halten av det inflammatoriska proteinet IL-6 i urin.

Tredje delarbetet visade att det var lika vanligt med positiva urinodlingar bland de som har respektive inte har ospecifika symtom vilket talar för att ospecifika symtom inte orsakas av bakterier i urinen. De som hade positiva urinodlingar hade också högre koncentrationer av IL-6 i urinen. Däremot var det inte någon skillnad i IL-6 koncentrationer mellan vårdtagare med bakterier i urinen som

hade respektive inte hade symtom. Därför är IL-6 inte användbart vid bedömning av patienter med ospecifika symtom och samtidigt bakterier i urinen. Urinstickor var inte heller användbara.

I det fjärde delarbetet har resistensdata för vanliga urinvägsantibiotika sammanställts från datainsamlingen 2012 och jämförts med resistensdata från datainsamlingen 2003. Överlag var resistenssiffrorna låga och det hade inte skett någon ökning mellan 2003 och 2012. Antibiotikabehandling under den senaste månaden liksom sjukhusvistelse under de senaste sex månaderna var däremot kopplade till högre resistenssiffror. För att bibehålla dessa internationellt sett låga resistenssiffror är det viktigt att inte använda antibiotika i onödan.

LIST OF PAPERS

This thesis is based on the following Papers, referred to in the text by their Roman numerals.

- I. Sundvall PD, Gunnarsson RK. Evaluation of dipstick analysis among elderly residents to detect bacteriuria: a cross-sectional study in 32 nursing homes. *BMC Geriatrics* 2009 Jul 27; 9:32.
- II. Sundvall PD, Ulleryd P, Gunnarsson RK. Urine culture doubtful in determining etiology of diffuse symptoms among elderly individuals: a cross-sectional study of 32 nursing homes. *BMC Family Practice* 2011 May 19; 12:36.
- III. Sundvall PD, Elm M, Ulleryd P, Mölsted S, Rodhe N, Jonsson L, Andersson B, Hahn-Zoric M, Gunnarsson RK. Interleukin-6 concentrations in the urine and dipstick analyses were related to bacteriuria but not symptoms in the elderly: a cross sectional study of 421 nursing home residents. Submitted.
- IV. Sundvall PD, Elm M, Gunnarsson RK, Mölsted S, Rodhe N, Jonsson L, Ulleryd P. Antimicrobial resistance in urinary pathogens among Swedish nursing home residents remains low: a cross-sectional study comparing antimicrobial resistance from 2003 to 2012. *BMC Geriatrics* 2014 Mar 13; 14(1):30.2.

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ABBREVIATIONS

ABU	Asymptomatic bacteriuria
UTI	Urinary tract infection
ESBL	Extended spectrum beta-lactamase
CFU/mL	Colony-forming units/mL
ELISA	Enzyme-linked immunosorbent assay
IL-6	Interleukin-6
PPV	Positive predictive value
NPV	Negative predictive value
EPV	Etiologic predictive value

DEFINITIONS IN SHORT

Nonspecific symptoms	Symptoms not specific to the urinary tract: fatigue, restlessness, confusion, aggressiveness, loss of appetite, frequent falls and a description of not being herself/himself.
Specific symptoms	Symptoms specific to the urinary tract, i.e. urinary frequency, dysuria and urgency.

1 INTRODUCTION

On the front page illustration we see Margaret, 85 years old. Margaret is living in a nursing home. She has been confused for the last few days. Her urine is odorous and the urine dipstick positive. The urine culture shows growth of *Escherichia coli* (*E. coli*). The attending nurse is now calling. She wonders if Margaret has a urinary tract infection (UTI). Would Margaret benefit from antibiotics? What do you think?

As many as half of elderly residents of nursing homes have asymptomatic bacteriuria (ABU). We are certain that ABU should not be treated with antibiotics. Has Margaret a symptomatic UTI? Or is there another reason for her confusion; and is the bacteria in her urine just a colonizer causing no symptoms, an ABU?

Nonspecific symptoms are the most common cause for suspecting UTI among elderly residents of nursing homes. The evidence base for such treatment is poor and the UTI diagnosis is all too often made in the absence of focal urinary tract symptoms. There is likely to be significant overtreatment with UTI antibiotics at nursing homes for the elderly due to this diagnostic uncertainty. A rational use of antibiotics is important due to the evolving threat of antibiotic resistance.

This common clinical problem, often leading to antibiotic treatment of doubtful value, was why I began my PhD studies. To sort this out it was necessary to investigate a possible correlation between nonspecific symptoms and findings of bacteria in the urine when considering ABU.

I am a general practitioner at a primary health care centre outside Borås, Sweden (Närhälsan Sandared). Besides PhD studies and clinical work I also work for Strama Västra Götaland. Strama is the Swedish Strategic Programme against Antibiotic Resistance. I have been carrying out my PhD studies at a Research and Development Centre in Södra Älvsborg (Närhälsan), the Sahlgrenska Academy at the University of Gothenburg and the National Research School in General Practice in Sweden.

1.1 Asymptomatic bacteriuria

Bacteriuria may represent a symptomatic UTI but could also be an ABU occurring regularly among elderly individuals [1-5]. There is overwhelming evidence that ABU should not be treated with antibiotics in an adult population

except for pregnant women and patients prior to traumatic urologic interventions with mucosal bleeding [6].

The presence of ABU among residents of nursing homes for the elderly varies between 25% and 50% for women and 15% and 40% for men [2, 7]. Thus a positive urine culture is a common finding, with or without clinical deterioration. Among nursing home residents, treatment of ABU does not lower the frequency of symptomatic infections or improve survival [6, 8-11]. Eradicating bacteriuria had no short-term effects on the severity of chronic urinary incontinence among nursing home residents [12]. Treatment of ABU is associated with increased adverse effects associated with antimicrobial treatment and reinfection by more antibiotic resistant bacteria [10]. There is a continuous problem with inappropriate treatment of ABU, and sustainable strategies are needed [13].

1.2 To treat or not to treat

UTI is the most commonly suspected bacterial infection among elderly residents of nursing homes [2, 14] often resulting in antibiotic treatment [15]. The high prevalence of ABU makes it difficult to know if new symptoms in residents with bacteriuria are caused by UTI, or if bacteriuria only represents an ABU [1, 5, 16, 17]. This is especially difficult in the presence of symptoms not specific to the urinary tract such as fatigue, restlessness, confusion, aggressiveness, loss of appetite or frequent falls.

There are different opinions on the possible association between nonspecific symptoms and UTI [1, 18-31]. This scientific uncertainty originates in the lack of a gold standard to determine if bacteriuria has to do with a new symptom, or if bacteriuria is merely an insignificant finding having nothing to do with the new symptom [1]. This is the main scientific problem to overcome when doing research on bacteriuria and symptoms among elderly residents of nursing homes.

1.2.1 The most common reasons for suspecting UTI

Contrary to common belief, dysuria, urinary urgency and frequency are not the main reasons for suspecting UTI among residents of nursing homes. The presenting symptom was dysuria in only 3.8%, urinary frequency in 1.5% and urinary urgency in 0% of the episodes of a suspected UTI in nursing home residents with advanced dementia [32]. Change in voiding patterns and urinary

tract specific symptoms were observed infrequently when nursing home staff members were asked: “What triggered suspicion of UTI?” [33].

Instead, changes in mental status such as lethargy, disorientation, restlessness, increasing irritability, aggressiveness, not being themselves, increased or new onset of confusion and delirium are the most common reasons for suspecting a UTI among residents of nursing homes [32, 34, 35]. These nonspecific symptoms (not specific for the urinary tract) can have many causes besides UTI [36]. Nonspecific symptoms and diagnostic uncertainty often lead to antibiotic treatments of dubious value [16, 35, 37, 38]. Symptoms such as dysuria, urinary urgency and frequency are consistent with the presentation of symptomatic UTI in other populations [39, 40]. However, physicians and nursing home staff members are relatively indiscriminate in diagnosing UTI in the absence of symptoms from the urinary tract in residents with bacteriuria [21, 29, 32]. Nonspecific symptoms or change in urine characteristics are not, in the absence of concomitant symptoms from the urinary tract, congruent with typical presentations of a symptomatic UTI [29].

The nurse is the key person in the identification of symptoms presumed associated with a UTI, playing a central role both in ordering urine cultures and communicating with physicians concerning the decision to prescribe antibiotics [35, 41].

Due to the evolving threat of antibiotic resistance [42] it is important to minimise unnecessary antibiotic treatment. A correct diagnosis is important to avoid a potentially harmful antibiotic treatment and possible delay of other diagnoses. Thus, it is important to clarify the association between symptoms, bacteriuria, dipstick urinalysis and other possible markers for UTI while considering the high prevalence of ABU, in order to improve the diagnostic procedure of a suspected lower UTI.

Urinary tract symptoms – not necessarily a UTI

Dysuria, urinary urgency and urinary frequency are symptoms from the lower urinary tract. These symptoms are often caused by a lower UTI among younger patients. There are many other causes of these symptoms among elderly patients, especially if symptoms are not of recent onset [43-45], and also a high prevalence of ABU among elderly residents. Thus, a urine culture is no gold standard even for the UTI diagnosis among elderly residents presenting with symptoms from the urinary tract.

It has been estimated that if a urine culture is positive in an institutionalized febrile elderly individual, without an indwelling urinary catheter and with no

local findings, only 16% of such episodes are attributable to a UTI [26]. Therefore, it is a safety risk to disregard other more plausible infections by taking for granted that fever and bacteriuria comprise a UTI.

1.2.2 Antibiotic use in nursing homes

Elderly people are prescribed substantially more antibiotics than younger people [46]. Residence in a nursing home setting is associated with even higher UTI antibiotic consumption [47]. A substantial proportion of elderly residents were on antibiotics in European point prevalence studies of antibiotic use [47-52]. The mean prevalence of antimicrobial therapy in 85 nursing homes throughout Europe in 2009 was 6.5% on a single day in April, and 5.0% on a single day in November [48]. Antibiotic therapy was also common in point prevalence studies in Canada [53] and Norway [54] as well as in a three-month survey of 58 nursing homes in Sweden in 2003 [14], and a six-month survey of 73 nursing homes in the U.S. in 2001/2002 [55]. In a Norwegian study, residents of nursing homes for the elderly used large amounts of antibiotics during a twelve-month follow-up [56].

Probably significant overtreatment

Patients in nursing home settings are frequently prescribed antibiotics on an empirical basis for presumed urinary tract infection when suffering from nonspecific symptoms [34, 35]. The evidence base for such treatment is poor [1, 16, 18], and all too often the UTI diagnosis is made in the absence of newly onset focal urinary tract symptoms [32, 37]. Therefore it is likely that a substantial proportion of these UTI antibiotics are of dubious value. Several studies report high rates of inappropriate antibiotic prescriptions in nursing homes [14, 32, 57, 58]. Despite evidence that ABU should not be treated, suspected UTI remains the most frequent reason for prescribing antibiotics in nursing homes for the elderly [2, 14], reflecting diagnostic uncertainty of the condition [1, 16, 38, 59, 60]. Treatment of ABU is also common in hospitals accounting for a substantial burden of inappropriate antimicrobial use in hospitals [61].

The Loeb minimum criteria, developed by a consensus conference in 2001, are minimum standards for initiation of antibiotics in long term care settings. These criteria require symptoms from the urinary tract for initiating antibiotics for suspected UTI in residents without indwelling urinary catheters [62]. Nonspecific signs, such as change in mental status are not part of the minimum criteria for initiating an antibiotic due to lack of specificity [62]. The adherence for the Loeb minimum criteria for UTI was only 10.2% (0.0-39%) in 12 North Carolina nursing homes in 2011 [58]. Evidence-based guidelines for antibiotic

initiation must be more widely adopted before any substantial gains from adherence are likely to be seen [58]. Improved knowledge for when to suspect a lower UTI would increase confidence in guidelines and quality in antibiotic stewardship in nursing homes for the elderly. Thus, it is important to study the role of bacteriuria in relation to nonspecific symptoms.

Pivmecillinam

Pivmecillinam is an oral antibiotic with excellent clinical efficacy in the treatment of uncomplicated UTI [63-65]. It has been used extensively in Scandinavia with few problems, but is not, however, widely used in other countries [63]. Pivmecillinam is well tolerated showing a low side-effect profile with little effect on the intestinal and vaginal flora of the host [63]. Furthermore, pivmecillinam was bacteriologically and clinically effective for treatment of lower UTI caused by Enterobacteriaceae producing extended spectrum beta-lactamase (ESBL) [66, 67].

1.2.3 Antimicrobial stewardship at nursing homes

Antimicrobial stewardship at nursing homes, including educational interventions targeting nursing home staff members and physicians, is important in the promotion of prudent antibiotic prescription [68-70]. Antimicrobial stewardship teams visiting long term care facilities and published guidelines were associated with a reduction in the usage of antimicrobials in long term care facilities in Finland [71]. Other programs focusing on specific aspects of UTI in long term care facilities have also been reported effective, however, without standardization their generalizability is uncertain [72]. A multifaceted intervention using diagnostic and therapeutic algorithms resulted in fewer antimicrobial prescriptions for a suspected UTI in a cluster randomized controlled trial in 24 nursing homes in Canada and the U.S. [73]. The proportions of any infection treated with antibiotics were significantly lower in the intervention group compared to controls in a cluster randomized controlled trial assessing an educational program targeting both nurses and physicians in Swedish nursing homes [74].

In order to further improve, unify and gain acceptance for evidence-based UTI guidelines, it is necessary to clarify the association between symptoms and bacteriuria while simultaneously considering the high prevalence of ABU among residents of nursing homes for the elderly.

Elderly people are more likely to experience adverse drug events of antibiotic treatment [75], however the main reason for avoiding unnecessary antibiotic treatment is increasing antibiotic resistance.

1.3 Antibiotic resistance in nursing homes

Antimicrobial resistance is on the rise and a cause of major concern in many countries [42, 76], as modern health care is dependent on effective antibiotics. Use of antibiotics is related to a higher prevalence of antibiotic resistant bacteria [77-83]. Elderly residents of nursing homes are prescribed a considerable amount of antibiotics. There are also several risk factors for colonization, infection and spreading of antibiotic resistant bacteria among elderly residents of nursing homes such as catheters, decubitus ulcers and wounds [84, 85]. There are some studies of antimicrobial resistance in uropathogens among elderly residents of nursing homes, however there were considerable differences in resistance rates between countries [86-91].

Antimicrobial resistance in urinary pathogens is still favourable in Sweden from an international perspective [46]. There was a low prevalence of ESBL-producing bacteria in faecal samples collected in Swedish nursing homes in 2008 [92]. However, between 2008 and 2010 ESBL faecal carriage increased both in the general population and at a university hospital in Sweden [93]. Resistance was generally low in 183 screening urine samples from Swedish residents of nursing homes in 2008-2010 [94]. There was a tendency towards higher antimicrobial resistance among strains isolated in 2010 from nursing home residents with indwelling bladder catheters compared to all urine strains at that laboratory [95].

It is important to frequently update information about the native prevalence of antimicrobial resistance in uropathogens among residents of nursing homes for the elderly, and to remain alert for significant changes. Any changes might affect empirical treatment of UTI and antibiotic stewardship in nursing homes. An updated estimate of the native prevalence of antimicrobial resistance in uropathogens among Swedish nursing home residents is needed.

1.4 Diagnostic methods

1.4.1 Dipstick urinalysis

Dipstick urinalysis is often the first measure in detecting bacteriuria [34, 41]. The diagnostic value of dipstick urinalysis has most often been evaluated in

younger populations. The clinical value of dipstick urinalysis could be quite different for elderly patients at nursing homes, since they have a higher prevalence of bacteriuria [2, 5, 7].

Sensitivity and specificity are of major interest for manufacturers of dipsticks, however these measures are of no interest to the physician making a clinical decision in a single case. The positive predictive value (PPV) and the negative predictive value (NPV), however, are of the utmost clinical importance to the physician. Predictive values are affected by the prevalence of bacteriuria [96].

When estimating sensitivity and specificity it is appropriate to present an interval estimate [97, 98]. This is rarely done in studies evaluating diagnostic tests [98]. The precision of predictive values, as with sensitivity and specificity, is dependent on sample size [98]. Therefore, it is also appropriate to use some kind of interval estimate for predictive values. Unfortunately, only a few studies evaluating dipstick urinalysis among the elderly has presented confidence intervals for PPV and NPV [99]. Other studies evaluating dipstick urinalysis among the elderly present confidence intervals only for sensitivity and specificity [19, 100], or no confidence intervals at all [101-107].

As Yule-Simpson's statistical paradox predicts, the outcome of analysing a single bacterial species might differ from analysing any bacterial species [108-110]. In such cases, results from analysing a single species are more appropriate than results from analysing any species. All previously published studies evaluating dipstick urinalysis of the elderly combine different bacterial species to any bacterial species when calculating sensitivity, specificity, PPV or NPV.

Several errors can occur during the testing procedure of urine dipsticks [111]. Timing and misalignment errors as well as subjectivity can be reduced by using a urine chemistry analyser [101, 111, 112]. Other studies showed only slightly improved reproducibility [113, 114] and no improvement in the analysis rate [113] by using mechanized methods. When urine tests are performed under routine conditions, results can be considerably lower even for simple tests such as nitrite, compared to optimal and standardized conditions achieved in most studies of the validity of urine tests [115]. Thus, the importance of analyser readings compared to visual readings of urine dipsticks in nursing homes for the elderly remains unclarified.

1.4.2 Urine culture

Provided bladder incubation time is sufficient it is unlikely that a patient has a UTI when the urine culture is negative. However, a positive urine culture only

indicates bacteria in the urine. The outcome of a urine culture cannot tell if a patient has a urinary tract infection [1]. Bacteria in the urine might represent a symptomatic UTI, but in the elderly it is most likely an ABU.

1.4.3 Interleukin-6

With the emergence of multidrug-resistant bacteria and the antimicrobial drug discovery pipeline currently running dry, it is important not to misinterpret bacteriuria as UTI and prescribe antibiotics when it actually represents ABU. Thus a complementary test to discriminate between symptomatic UTI and ABU is needed. The combination of bacteria in the urine and pyuria cannot differentiate between UTI and ABU in residents of nursing homes [116]. A continued search for additional biomarkers to diagnose UTI is important [29, 117].

The cytokine Interleukin-6 (IL-6) is a mediator of inflammation playing an important role in the acute phase response and immune system regulation [117, 118]. The biosynthesis of IL-6 is stimulated by e.g. bacteria [118]. After intravesical inoculation of patients with *E. coli*, all patients secreted IL-6 into the urine [119]. There had to be $\geq 10^5$ colony-forming units (CFU)/mL to completely stimulate IL-6 secretion in urine. However, serum concentrations of IL-6 did not increase, suggesting a dominance of local IL-6 production [119].

The cytokine responses in IL-6 and IL-8 to UTI in children varied with the severity of infection, and those with ABU had lower concentrations of these cytokines in the urine [120]. The clear correlation between concentrations of IL-6 and IL-8 and disease severity suggested that cytokine measurements can be used to further discriminate between UTI and ABU [120]. A symptomatic lower UTI is assumed associated with more severe inflammation in the bladder compared to an ABU. Further studies of biomarkers for the diagnosis of UTI is particularly important in the elderly [117]. Previous studies suggested that concentrations of IL-6 in the urine may be valuable in discriminating between ABU and cystitis in the elderly, however, this needs evaluation in a larger study among the elderly [17, 121]. It is important to evaluate if IL-6 could be such a test, as it is crucial to find a diagnostic tool to differentiate between ABU and cystitis.

2 AIM

The general aim of this thesis was to clarify the association between symptoms, bacteriuria, dipstick urinalysis and urine IL-6 among nursing home residents to improve the diagnostic procedure of a suspected lower UTI.

2.1 Specific aims of this thesis

The specific aims of this thesis were:

- To document sensitivity, specificity, PPV and NPV with 95% confidence intervals for the ability of bedside dipstick urinalysis to detect bacteriuria among residents of nursing homes for the elderly.
- To compare visual readings of urine dipsticks with a urine chemistry analyser.
- To investigate the relationship between bacteria in the urine and new or increased restlessness, fatigue, confusion, aggressiveness, loss of appetite, frequent falls, not being herself/himself, dysuria, urinary urgency and frequency in residents of nursing homes for the elderly, when statistically considering the high prevalence of ABU in this population.
- To investigate the association between laboratory findings of bacteria in the urine, elevated IL-6 concentrations in the urine, dipstick urinalysis and new or increased symptoms in residents of nursing homes for the elderly.
- To describe antimicrobial resistance rates in uropathogens among residents of Swedish nursing homes for the elderly in 2012 and compare these to the 2003 rates.
- To determine if antibiotic treatment within the previous month or hospitalization within the previous six months predicted higher resistance rates in uropathogens among residents of nursing homes for the elderly.

3 PATIENTS AND METHODS

During two consecutive two-week periods in February and March 2003 a study protocol was completed and single urine specimens collected from all included residents of 32 participating nursing homes. This data was used for a study of dipstick urinalysis (Paper I) and a study of nonspecific symptoms and bacteriuria (Paper II), (Table 1).

From January to March 2012, a study protocol was completed and single urine specimens collected from all included residents of 22 nursing homes. This data was used for a study of IL-6 (Paper III) and an antimicrobial resistance study (Paper IV) where resistance rates were also compared to results from the data gathered in 2003 (Table 1).

Table 1. A brief summary of the most important characteristics of the Papers

	Paper I	Paper II	Paper III	Paper IV
Data gathering	2003	2003	2012	2012 and 2003
Urine specimens	Voided	Voided	Voided	Voided + urinary catheter
Urine cultures ¹	Significant and sparse growth	Significant and sparse growth	Significant growth only	Significant and sparse growth
Antimicrobial resistance				Among urinary pathogens
Nonspecific symptoms ²		Duration: < 3 months	Duration: < 1 month	
Urinary tract symptoms		Duration: < 3 months	Duration: < 1 month	
Dipstick urinalysis ³	Visually and analyser read		Visually read	
Interleukin-6			In the urine	

¹Sparse and significant growth is defined in section 3.4.2. ² Symptoms not specific to the urinary tract were; fatigue, restlessness, confusion, aggressiveness and a description of not being herself/himself in 2003 as well as loss of appetite and frequent falls in 2012.

³Dipstick urinalysis regarding nitrite and leukocyte esterase.

In 2003, the 32 participating nursing homes were located in four municipalities in south-western Sweden. Two of these municipalities had 22 participating nursing homes in 2012. The nursing homes were located in both urban and rural areas.

The studies were approved by the Regional ethical review board of Gothenburg University (D-nr Ö 410-02 and 578-11).

3.1 Inclusion criteria

Residents of the participating nursing homes for the elderly, regardless of UTI symptoms were invited to participate. Those accepting participation were included if they met the following inclusion criteria:

- Permanent residence in nursing homes for the elderly (regardless of gender)
- Presence at a nursing home for the elderly during the study
- Participation approval
- In Papers I-III: No indwelling urinary catheter (only voided urine specimens were collected). In Paper IV: Both voided urine specimens and specimens from indwelling urinary catheters were collected
- Sufficiently continent to leave a voided urine specimen (unless the resident had an indwelling urinary catheter in Paper IV)
- Residents with dementia were included if cooperative when collecting urine samples
- No urostomy
- No regularly clean intermittent catheterisation
- Not terminally ill
- No ongoing peritoneal- or haemodialysis

3.2 Statement of consent

Residents were informed of the studies verbally and in writing. Informed approval for participation in the studies was collected from decision-capable individuals choosing to participate in the study. However, a considerable number of participants consisted of residents with varying degrees of dementia. If the resident was incapable of understanding information and thereby possessing a reduced decision capability, these residents only participated so long as they did not oppose participation and under the condition that

appointed representatives or relatives did not oppose their participation after having partaken of the study information. This procedure was approved by the Regional ethical review board of Gothenburg University.

3.3 Study protocol and symptoms

In addition to collecting the urine sample, the attending nurse made an entry in the study protocol for each included resident whether having any of the following symptoms, newly onset or increased within the last three months and still present when the urine specimen was obtained; fatigue, restlessness, confusion, aggressiveness, not being herself/himself, fever, dysuria and urinary urgency (Papers I-II). Depending on how long symptoms had persisted or increased, they were divided into the following three groups: less than one week, more than one week but less than one month, or more than one month but less than three months. Any ongoing and/or previous antibiotic treatment within the last month and/or diabetes mellitus were also registered.

For Papers III-IV the attending nurse also registered the following symptoms; loss of appetite, frequent falls and urinary frequency as well as dementia, overnight admissions to hospital within the last six months and any antibiotic treatment within the last six months.

Throughout this thesis, the label nonspecific symptoms will be used for symptoms not specific to the urinary tract: fatigue, restlessness, confusion, aggressiveness, loss of appetite, frequent falls and a description of not being herself/himself in some other way.

The attending nurses at the nursing homes were carefully instructed to register presence or absence of symptoms in the study protocol before collecting urine specimens and performing dipstick urinalysis. Thus the evaluation of symptoms was not influenced by the results of the urine tests.

To avoid presence of symptoms influencing what day the study protocol was completed and urine specimen collected, there was a predetermined date for collection of the urine sample from each included resident (Papers III- IV). For Papers I and II, each nursing home had to complete data collection within two weeks.

3.4 Urine specimens and laboratory tests

Personnel at the nursing homes were instructed to collect a mid-stream morning urine sample, or a voided urine specimen with as long a bladder incubation time as possible. For the purpose of Paper IV, a single urine specimen was also collected from residents with indwelling urinary catheters. Body temperature was measured by ear thermometer (Paper III).

3.4.1 Dipstick urinalysis

Visual readings (Papers I-IV)

Immediately after collecting urine samples, dipstick urinalysis was carried out at the nursing home. Visual reading of the urine dipstick Multistix 5 was performed for the detection of nitrite and leukocyte esterase. The Multistix 5 Reagent Strips were manufactured by Bayer HealthCare Diagnostics Division (Papers I-II) and by Siemens Healthcare Laboratory Diagnostics (Papers III-IV) as Bayer Diagnostics was acquisitioned by Siemens Medical Solutions in 2006.

Analyser readings (Paper I)

In paper I, a second urine dipstick (also Multistix 5) was analysed for the detection of nitrite and leukocyte esterase, with the urine chemistry analyser Clinitek 50 (Bayer HealthCare Diagnostics Division) [122]. The nursing home personnel were instructed by a representative from the manufacturer in the handling of the analyser Clinitek 50 and the Multistix 5 reagent strip. For visual readings not to be influenced by the result of analyser readings, the attending nurses were carefully instructed to register the results of the visual readings before using the urine chemistry analyser.

3.4.2 Urine cultures

Urine specimens were cultured at the microbiology laboratory at the Södra Älvsborg Hospital in Borås, Sweden using clinical routine procedure. The urine specimens were chilled before transport and usually arrived at the laboratory within 24 hours. As in clinical routine, the laboratory was provided information on the outcome of the dipstick urinalysis as well as information on any urinary tract specific UTI symptoms from the attending nurse.

Culture techniques and cut-off points

The microbiology laboratory fractionated 10 µl urine on the surfaces of two plates; a cystine-lactose-electrolyte deficient agar (CLED) and a Columbia blood agar base. Plates were incubated overnight (minimum 15 h) at 35-37 °C. CLED plates were incubated in air, and Columbia plates were incubated in 5%

CO₂. The latter was further incubated for 24 hours if no growth occurred after the first incubation.

Growth of bacteria was considered significant if the number of colony-forming units (CFU)/mL was $\geq 10^5$. However, at signs of possible UTI such as a positive nitrite dipstick, leukocyte esterase dipstick >1 , fever, dysuria, urinary urgency or frequency, the cut-off point was $\geq 10^3$ for patients with a growth of *E. coli* and for male patients with *Klebsiella* species (spp.) and *Enterococcus faecalis* (*E. faecalis*). For symptomatic women harbouring the two latter species the cut-off level was set at $\geq 10^4$. At these lower cut-off points and with no specific symptoms or signs of a possible UTI, the urine cultures were classified as sparse growth. However, it is important to notice that nonspecific symptoms did not influence cut-off levels for CFU/mL in the urine cultures. For the purpose of Papers I-II and IV, both significant and sparse growth were defined as a positive urine culture. In the IL-6 study (Paper III), only significant growth was considered a positive urine culture.

In case of several detected species *E. coli* was selected in favour of secondary pathogens such as *Klebsiella* spp. and in case of several *E. coli*, the most prevalent isolate was selected (Papers III-IV). Growth of a mixed flora was classified as a negative urine culture (Papers I-IV).

Antimicrobial susceptibility

The antimicrobial susceptibility of bacteria was determined according to the disc diffusion method described by the Swedish Reference Group for Antibiotics (SRGA) at the time. In 2012, antimicrobial susceptibility tests followed guidelines and breakpoints proposed by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) for the standardized disk diffusion test [123]. In 2003 nalidixic acid was used as a screening disk for any quinolone resistance; in 2012 isolates were only tested for ciprofloxacin resistance according to national guidelines. Bacterial isolates with suspected ESBL production were confirmed as ESBL-producing bacteria by the reference laboratory at the Swedish Institute for Communicable Disease Control.

3.4.3 IL-6 and creatinine in urine

Measurements of the concentrations of IL-6 in the urine were performed with enzyme-linked immunosorbent assay (ELISA) using a commercial kit (Quantikine HS ELISA, High Sensitivity) [124] according to instructions from the manufacturer (R&D Systems, Abingdon, Oxford, UK) at the clinical

immunology laboratory at the Sahlgrenska University Hospital in Gothenburg, Sweden.

Concentrations of creatinine in the urine were analysed by the automated general chemistry analyser UniCel[®] Dx_C 800 Synchron[®] Clinical System, according to instructions from the manufacturer (Beckman Coulter), at the clinical chemistry laboratory at the Södra Älvsborg Hospital in Borås, Sweden.

3.5 Statistical analysis

The most important statistical tests are summarised in Table 2.

Table 2. A brief summary of the most important statistical tests

	Paper I	Paper II	Paper III	Paper IV
Pearson's chi-square test			X	X
Fisher's exact test			X	X
Mann-Whitney test			X	
Logistic regression	X	X	X	X
Etiologic predictive value		X		
Sensitivity and specificity	X			
PPV and NPV ¹	X			
Kappa coefficient	X			

¹Positive predictive value (PPV) and negative predictive value (NPV).

3.5.1 Paper I

Sensitivity, specificity, PPV and NPV were calculated for nitrite and leukocyte esterase separately using a urine culture as the gold standard. Similar estimates were calculated for combinations of nitrite and leukocyte esterase. Ruling in

or ruling out bacteriuria was considered possible when a point estimate of PPV/NPV was $\geq 85\%$ with a lower confidence interval of $\geq 80\%$.

The association between dipstick findings and urine cultures was further evaluated by logistic regression to avoid confounding factors such as sex and age leading to false conclusions. Presence or absence of a potentially pathogenic bacterium in urine culture was used as the dependent variable while outcome of dipstick, age and gender were independent variables.

Agreement between visual and analyser readings of dipsticks was calculated by Kappa coefficient. Kappa coefficient with confidence intervals was calculated using CIA (Confidence Interval Analysis) version 2.1.2 (Bryant, University of Southampton, England) [125].

Epi Info version 3.3.2 (Windows version) (CDC, Atlanta, USA) was used for logistic regressions. Calculations for sensitivity, specificity, PPV and NPV were made in Microsoft Office Excel 2003 version 11.8 SP2.

3.5.2 Paper II

Newly onset or increased symptoms during the last three months (and still present when urine specimen was obtained) were also divided into the following three intervals: less than one week, one week or more but less than one month, one month or more but less than three months.

To evaluate the statistical correlation between bacteriuria and presence of a symptom on a group level, logistic regressions were performed. The symptom was used as dependent variable and the outcome of the urine culture, age and gender as independent variables. One regression was made for each symptom and duration interval respectively. Finally, one regression was also made for each symptom merging time intervals to all those occurring within three months. If no relevant differences were found, only the latter was presented.

To estimate clinical relevance of statistical correlations, the positive and negative etiologic predictive value (EPV) [126] was calculated to evaluate the probability of a positive/negative culture to rule in or out that a symptom in a single individual is associated with a bacterial finding. EPV was considered clinically useful if their point estimate was $\geq 75\%$ with a lower 95% confidence interval $\geq 50\%$.

Epi Info version 3.3.2 (Windows version) (CDC, Atlanta, USA) was used for the statistical analyses. EPV with confidence intervals was calculated using the EPV-Calculator version 1.12 [127].

3.5.3 Paper III

Symptoms considered in Paper III were newly onset or increased symptoms during the last month and still present when the urine specimen was obtained. Symptoms remaining unchanged for one month or more were not taken into account.

The first objective was to clarify whether the concentrations of IL-6 in the urine or urine dipsticks differed between residents with or without bacteriuria. Concentrations of IL-6 in the urine and outcome of urine dipstick analyses were compared between residents with positive and negative urine cultures, irrespective of symptoms, using the Mann-Whitney test for IL-6 (due to skewed data) and the Pearson's chi-square test for dipsticks.

The second and third objective was to clarify whether a symptom correlated to bacteriuria or antibiotic usage. The prevalence of bacteriuria or use of antibiotics was compared between residents with or without symptoms using Pearson's chi-square test. Fisher's exact test was used in case of small numbers.

The fourth objective was to clarify if the concentrations of IL-6 in the urine or outcomes of urine dipstick analyses differed depending on symptoms in residents with bacteriuria. Concentrations of IL-6 in the urine or outcome of dipstick analyses were compared between bacteriuric residents with or without symptoms using Mann-Whitney's test for IL-6 (due to skewed data) and Pearson's chi-square test for dipsticks.

The fifth objective was to correlate factors with symptoms while adjusting for covariates. A cut-off was used to construct a dichotomous variable covering approximately 20% of the highest IL-6 concentrations (≥ 5 ng/L). A similar dichotomous variable was constructed for urine dipstick leukocyte esterase where $\geq 3+$ was considered positive. Forward stepwise (conditional) logistic regressions were performed where the condition for entry was 0.050 and for removal 0.10. Variables that served well for the overall prediction were also kept in the model. Zero order correlations between independent variables were checked and correlations > 0.6 were not allowed. The independent variables, all but age being dichotomous, were; urine culture, IL-6 in the urine, leukocyte esterase dipstick, nitrite dipstick, antibiotics during the last month, age, gender, and presence of diabetes mellitus or dementia.

IBM SPSS Statistics version 21 was used for statistical analysis.

3.5.4 Paper IV

Differences between bacterial species and resistance patterns between 2003 and 2012 were analysed by Pearson's chi-square, and when appropriate, Fisher's exact test.

To evaluate the impact of previous antibiotic treatments during the last month on antimicrobial resistance rates, adjusted for age and gender, logistic regressions were performed for those with *E. coli* in voided urine specimens collected in 2003 and 2012. The outcome of antimicrobial susceptibility testing was used as the dependent variable and age, gender and antibiotic treatments during the previous month as independent variables. One regression was made for each antibiotic commonly used to treat UTI.

To evaluate the impact of hospitalization during the previous six months on antimicrobial resistance rates, adjusted for age, gender and antibiotic treatments during the previous six months, logistic regressions were performed for those with *E. coli* in voided urine specimens collected in 2012. The outcome of antimicrobial susceptibility testing was used as the dependent variable, and any hospitalization and any antibiotic treatment during the previous six months, as well as age and gender, as independent variables. One regression was made for each antibiotic commonly used to treat UTI and finally for any antimicrobial resistance tested. Cramer's V was calculated to evaluate any correlation between "any hospitalization" and "any antibiotic treatment" during the previous six months.

IBM SPSS Statistics version 21 was used for statistical analysis.

4 RESULTS

Papers I, II and IV have been published. Paper III is a submitted manuscript not as yet published. As this thesis will be available electronically, complete result tables for Paper III are not included in this thesis to avoid problems with future publication. However, these tables can be found in the attached manuscripts for Paper III within the hard copy of the printed thesis. The numbering for these attached tables within the manuscripts are “Paper III, Table 1” and so on.

4.1 Characteristics of studied populations

Papers I and II were based on the studied population in 2003. The studied population in 2012 was used for Papers III and IV. Paper IV also compared change in bacterial growth and antimicrobial resistance between 2003 and 2012.

4.1.1 Studied population in 2003

In 2003, 751 of 1187 residents in 32 nursing homes fulfilled the inclusion criteria, and 655 (87%) accepted participation (Figure 1). Voided urine specimens were provided from 651 individuals, 482 (74%) women and 169 (26%) men.

When the urine specimens were collected, 26/651 (4.0%) were undergoing antibiotic treatment. Another 61/651 (9.4%) had no ongoing antibiotic treatment when the urine specimens were collected but had received antibiotics during the previous month. Antibiotic treatment history was, however, unknown for 12/651 (1.8%).

Women (mean age 86 years, SD 7.4, range 46-102) were slightly older than men (mean age 82 years, SD 7.8, range 54-99) ($p < 10^{-6}$). Among participating residents 100/651 (15%) suffered from diabetes mellitus.

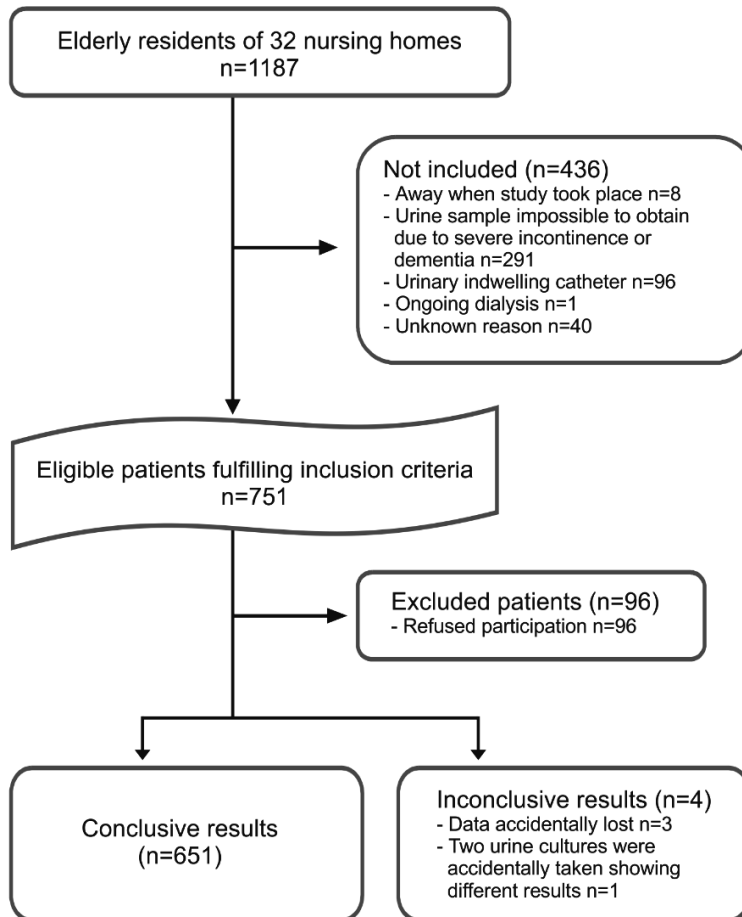


Figure 1. Participant flow chart in 2003, Papers I, II and part of Paper IV.

4.1.2 Studied population in 2012

Residents with indwelling urinary catheters were included in the study of antimicrobial resistance (Paper IV). However, residents with an indwelling urinary catheter were not included in Paper III studying symptoms, bacterial

findings and IL-6 in the urine. Data was collected in the first three months of 2012.

Paper III

Inclusion criteria were fulfilled by 676 of 901 residents in 22 nursing homes, and 425 (63%) accepted participation (Figure 2). Of the participating residents 295 (70%) were women and 126 (30%) were men. Voided urine specimens for culture and symptom forms were provided from 421 residents.

When urine specimens were collected, 18/421 (4.3%) were undergoing antibiotic treatment. Another 29/421 (6.9%) had no ongoing antibiotic treatment when the urine specimen was collected but had received antibiotics during the previous month.

Women (mean 87 years, SD 6.4, range 63-100) were slightly older than men (mean 85 years, SD 7.1, range 65-100) ($p=0.0053$). Among participating residents 56/421 (13%) suffered from diabetes mellitus and 228/421 (54%) had dementia. Measure of body temperature was conclusive in 399/421 residents; none of these residents had a body temperature $\geq 38.0^\circ$ Celsius.

Paper IV

2012

Inclusion criteria were fulfilled by 735 of 901 residents in 22 nursing homes and 484 (66%) accepted participation (Figure 3). Of the participating residents 321 (67%) were women and 159 (33%) men. Urine specimens were provided from 480 residents (421 voided urine specimens and 59 urine specimens from indwelling urinary catheters).

When the urine specimens were collected, 23/480 (4.8%) had ongoing antibiotic treatment. Another 45/480 (9.4%) had no ongoing antibiotic treatment when the urine specimens were collected, but had received antibiotics during the previous month.

Women (mean 87 years, SD 6.6, range 62-100) were slightly older than men (mean 85 years, SD 7.0, range 65-100) ($p=0.010$). The presence of new or increased urinary symptoms occurring within the last week was for dysuria 5/480 (1.0%), urinary urgency 6/480 (1.3%), and urinary frequency 2/480 (0.42%). Of the participating residents 71/480 (15%) suffered from diabetes mellitus and 253/480 (53%) had dementia.

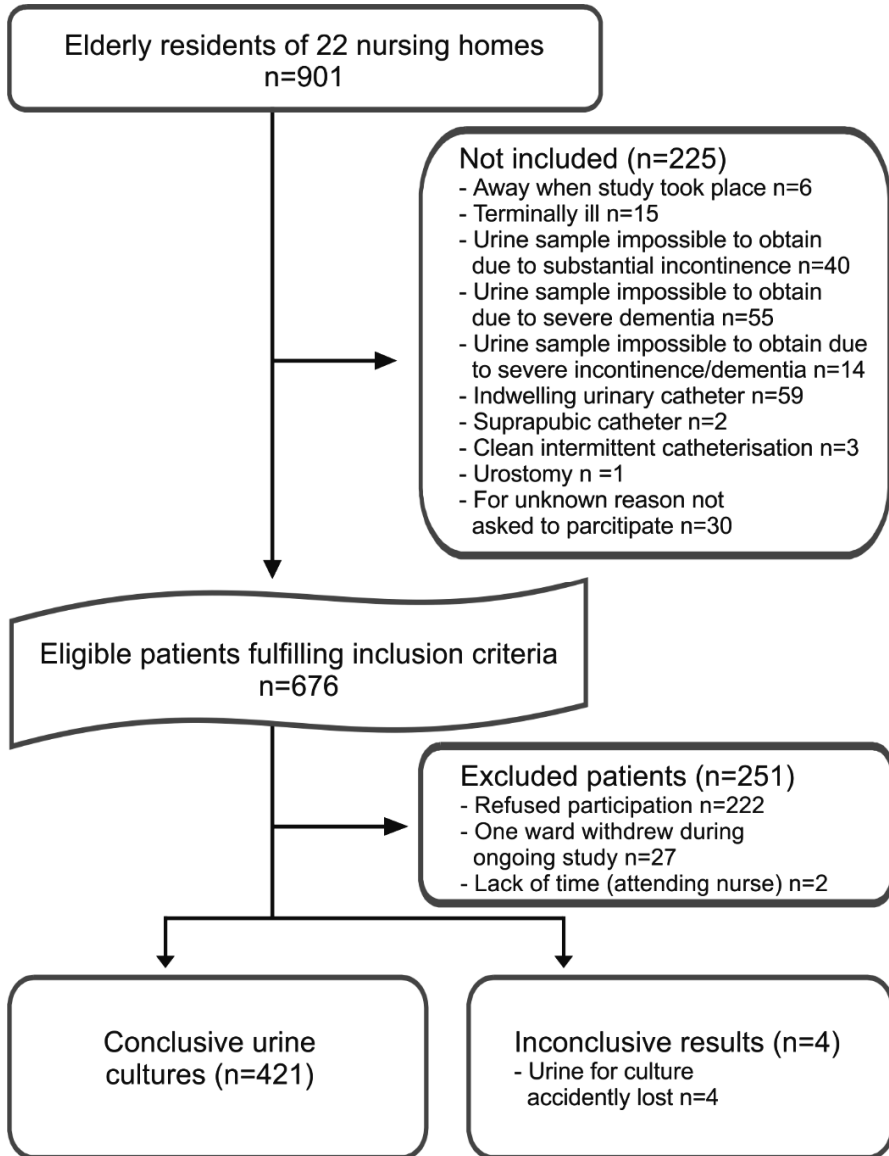


Figure 2. Participant flow chart in 2012, Paper III.

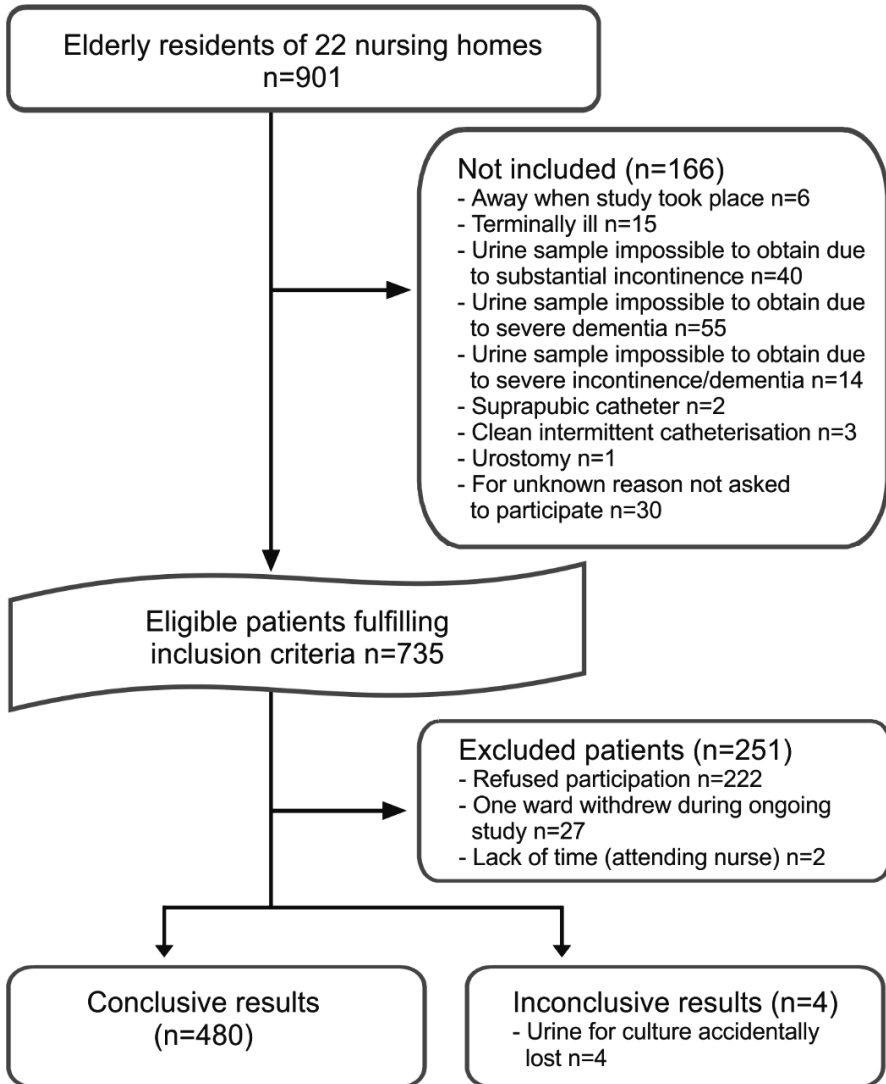


Figure 3. Participant flow chart in 2012, Paper IV.

Comparing populations in 2003 and 2012 (Paper IV)

When comparing the studied populations in 2003 and 2012 men's mean age was 3.1 years higher in 2012 compared to 2003 ($p=0.00016$) with a trend towards a higher mean age among women, however not significant (0.92 year, $p=0.073$). The proportion of men was higher in 2012, 33% compared to 26% in 2003 ($p=0.0087$).

4.2 Bacterial findings

In 2003 (Papers I-II), 32% (207/651) of voided urine cultures showed significant or sparse growth of potentially uropathogenic bacterial species (Table 3). The most common findings were; *E. coli* in 22% (143/651) of the residents, *Klebsiella* spp. in 3.8% (25/651) of the residents and *Enterococcus faecalis* (*E. faecalis*) in 2.6% (17/651) of the residents. Other species had very low prevalence among the nursing home residents ($\leq 0.8\%$ for each species).

Table 3. Bacterial growth in urine cultures in 2003 and 2012 (Papers I-IV)

	Voided urine 2003 N=651	Voided urine 2012 N=421	Urinary catheter 2012, N=59
Negative culture	68% (444/651)	65% (274/421)	54% (32/59) ¹
Sparse growth	2.9% (19/651)	2.9% (12/421)	1.7% (1/59)
Significant growth	29% (188/651)	32% (135/421)	44% (26/59)
Sparse + significant	32% (207/651)	35% (147/421)	46% (27/59)

¹There were mixed growths in all but one urine culture obtained from urinary catheters, classified as negative.

In 2012 (Papers III-IV), there was significant growth of potentially pathogenic bacteria in 32% (135/421) of voided urine specimens (Tables 3-4). There was sparse growth of potentially pathogenic bacteria in an additional 2.9% (12/421) of voided urine specimens (Tables 3-4). *E. coli* was by far the most common finding, present in 81% (109/135) of positive voided urine cultures with significant growth. *Klebsiella* spp. were the second most common finding, present in 8.1% (11/135) of positive cultures with significant growth. *Proteus* spp. were present in 3.0% (4/135) of positive cultures with significant growth. Other species had very low prevalence's, $\leq 1.5\%$ of positive urine cultures for each species.

In 2012 (Paper IV) 46% (27/59) of the cultures of urine specimens obtained from indwelling urinary catheters were classified as positive (Table 3). There were growths of a mixed bacterial flora in all but one of the cultures obtained from urinary catheters, classified as negative. The bacterial findings in positive urine cultures obtained from urinary catheters were; *E. coli* 48% (13/27), *Klebsiella* spp. 37% (10/27), *Proteus mirabilis* 11% (3/27) and *Enterobacter* spp. 3.7% (1/27).

Gender differences in bacterial findings

The proportion of positive urine cultures (sparse and significant growth) from voided urine was more common among women than men; 38% (184/482) in

women versus 14% (23/169) in men ($p < 10^{-6}$) in 2003, and 45% (134/295) in women versus 10% (13/126) in men ($p < 10^{-6}$) in 2012.

Findings of *E. coli* in positive urine cultures from voided urine were more common among women than men; 73% (134/184) versus 39% (9/23), $p = 0.00098$ in 2003 and 82% (110/134) versus 54% (7/13), $p = 0.027$ in 2012.

Similarities and differences between 2003 and 2012

There was no significant difference in percentage of positive urine cultures (sparse and significant growth) from voided urine samples in 2003 and 2012; 32% versus 35% ($p = 0.29$). However, there was a significantly higher proportion of *E. coli* in positive urine cultures from voided urine samples in 2012 compared to 2003; 117/147 (80%) in 2012 versus 143/207 (69%) in 2003, $p = 0.027$. Table 4 provides an overview of sparse and significant growth of each potentially pathogenic bacterial species in voided urine in 2003 and 2012 (Papers I-IV).

Table 4. Bacterial growth in positive urine cultures from voided urine in 2003 and 2012 (Papers I-IV)

	===== 2003 =====			===== 2012 =====		
	Sparse (n=19)	Significant (n=188)	Total ¹ (n=207)	Sparse (n=12)	Significant (n=135)	Total ¹ (n=147)
<i>Escherichia coli</i>	6.8% (14)	62% (129)	69% (143)	5.4% (8)	74% (109)	80% (117)
<i>Klebsiella</i> species	0.48% (1)	11% (22)	11% (23)	0	7.5% (11)	7.5% (11)
<i>Enterococcus faecalis</i>	1.0% (2)	7.2% (15)	8.2% (17)	0	0.68% (1)	0.68% (1)
<i>Enterococcus faecium</i>	0.48% (1)	1.4% (3)	1.9% (4)	0	0	0
<i>Proteus</i> species	0	0	0	0	0.68% (1)	0.68% (1)
<i>Proteus mirabilis</i>	0	1.4% (3)	1.4% (3)	0	1.4% (2)	1.4% (2)
<i>Proteus vulgaris</i>	0	0.48% (1)	0.48% (1)	0	0.68% (1)	0.68% (1)
<i>Enterobacter</i> species	0	1.9% (4)	1.9% (4)	0	1.4% (2)	1.4% (2)
<i>Pseudomonas aeruginosa</i>	0	1.4% (3)	1.4% (3)	0	0	0
<i>Citrobacter</i>	0	0	0	0	1.4% (2)	1.4% (2)
<i>Serratia liquefaciens</i>	0	0	0	0	0.68% (1)	0.68% (1)
<i>Staphylococcus aureus</i>	0	0	0	0.68% (1)	0.68% (1)	1.4% (2)
Coagulase-negative staphylococci	0	1.9% (4)	1.9% (4)	0	0.68% (1)	0.68% (1)
Alpha-hemolytic streptococci	0	1.0% (2)	1.0% (2)	0	1.4% (2)	1.4% (2)
Group G beta-hemolytic streptococcus	0	0.48% (1)	0.48% (1)	1.4% (2)	0	1.4% (2)
Group C beta-hemolytic streptococcus	0	0	0	0.68% (1)	0	0.68% (1)
Group B beta-hemolytic streptococcus	0.48% (1)	0.48% (1)	1.0% (2)	0	0.68% (1)	0.68% (1)

¹ Growth of bacteria in all positive urine cultures, % (n).

4.3 Symptoms and bacteriuria

In 2003 (Paper II), fatigue, restlessness and confusion were the most common symptoms (Table 5). The proportion of positive urine cultures (significant and sparse growth) among residents with symptoms are presented in Table 5. Combined symptoms were uncommon. The four most prevalent combined symptoms were restlessness and fatigue 1.7% (11/651), fatigue and confusion 1.1% (7/651), fatigue and urinary urgency 0.92% (6/651) and restlessness and confusion 0.92% (6/651).

Table 5. Prevalence of symptoms and positive urine cultures 2003 (Paper II)

	Prevalence of symptoms ¹ 0-3 months	Proportion of positive urine cultures; significant + sparse growth
Fatigue	12% (80/651)	41% (33/80)
Restlessness	9.1% (59/651)	39% (23/59)
Confusion	7.5 % (49/651)	45% (22/49)
Aggressiveness	4.3% (28/651)	39% (11/28)
Not being herself/himself	2.3% (15/651)	60% (9/15)
Dysuria	1.8% (12/651)	42% (5/12)
Urinary urgency	5.5% (36/651)	42% (15/36)
Fever	0.31% (2/651)	50% (1/2)
All residents (n=651)		32% (207/651)

¹ Symptoms commencing at any time during the preceding three months and still present.

The prevalence of new or increased symptoms, occurring during the last month and still present when urine specimens were obtained in 2012 (Paper III) are presented in Table 6. The proportion of positive urine cultures (only significant growth) among residents with symptoms are also presented in Table 6.

There were no significant differences in the proportion of positive urine cultures in 2012 among those with or without nonspecific symptoms, however there were less positive urine cultures among residents with urinary frequency (Table 6). This is presented in more detail, p-values included, in Paper III, Table 1. Residents with some of the symptoms had a higher prevalence of antibiotic treatments during the last month (Paper III, Table 2).

Table 6. Prevalence of symptoms and positive urine cultures 2012 (Paper III)

	Prevalence of symptoms ¹ 0-1 month	Proportion of positive urine cultures (only significant growth)
Fatigue	11% (48/421)	31% (15/48)
Restlessness	5.5% (23/421)	26% (6/23)
Confusion	5.2 % (22/421)	14% (3/22)
Aggressiveness	5.0% (21/421)	19% (4/21)
Loss of appetite	5.2% (22/421)	18% (4/22)
Frequent falls	5.2% (22/421)	23% (5/22)
Not being herself/himself	4.3% (18/421)	39% (7/18)
Having <i>any</i> of the nonspecific symptoms	20% (85/421)	31% (26/85)
Having <i>none</i> of the nonspecific symptoms	80% (336/421)	32% (109/336)
Dysuria	2.1% (9/421)	11% (1/9)
Urinary urgency	3.6% (15/421)	33% (5/15)
Urinary frequency	2.4% (10/421)	0% (0/10)
All residents (n=421)		32% (135/421)

¹ Symptoms commencing at any time during the preceding month and still present.

4.4 Predictors of symptoms

Paper II

Not being herself/himself occurring within 3 months correlated statistically on a group level with findings of *E. coli* (Table 7) and any bacteria (Table 8), adjusted for age and gender. Fever acquired within 1 week correlated with findings of *Klebsiella* spp. (adjusted odds ratio 45 with 95% CI 2.0-980, p=0.016). Confusion or fatigue that occurred or changed within 3 months correlated with findings of any bacteria (Table 8).

If duration of symptoms includes all patients with new or changed symptoms within 3 months, the EPV suggests that none of these statistical findings had any clinical relevance (Tables 7-8). However, when analysing duration of symptoms separately, EPV showed that in a patient where not being herself/himself for the preceding period of >1 month and <3 months, growth of *E. coli* was with 96% (51-100%) probability associated with this symptom. For the purpose of Paper II, both significant and sparse growth was defined as a positive urine culture. However, if only considering significant growth as a

positive urine culture, the odds ratio for *E. coli* to predict not being herself/himself for the preceding period of >1 month and <3 months was not significant, odds ratio (OR) 4.6 (0.75-28; p=0.098), adjusted for age and gender. Subsequently the positive EPV was not clinically relevant if only considering significant growth of *E. coli*, 86% (0-100%). For the purpose of Paper III, we only analysed newly onset or increased symptoms during the last month and still present when the urine specimen was obtained. If, despite this, calculating the odds ratio for significant and sparse growth of *E. coli* to predict not being herself/himself for the preceding period of >1 month and <3 months from data gathered in 2012, the OR (adjusted for age and gender) was not significant, OR 0.25 (0.030-2.1; p=0.20). Nor was it significant if only considering significant growth as a positive urine culture, adjusted OR 0.28 (0.034-2.3; p=0.24). It was not relevant to calculate EPV (further discussed in section 5.3.5 EPV) since these logistic regressions did not identify any significant odds ratios.

No other subset of duration showed any clinically relevant EPV in Paper II.

Table 7. Odds ratios and probabilities that findings of Escherichia coli in the urine¹ are associated with symptoms (Paper II)

	Prevalence of symptom ²	Statistical correlation ³	Clinical relevance ⁴	
		Adjusted odds ratio (95% CI; p-value)	Positive EPV % (95% CI)	Negative EPV % (95% CI)
Restlessness	9.1% (59/651)	1.4 (0.76-2.6; p=0.28)	34 (0-72)	99 (94-100)
Fatigue	12% (80/651)	1.7 (0.99-2.9; p=0.057)	40 (0-73)	98 (94-100)
Confusion	7.5% (49/651)	1.8 (0.96-3.6; p=0.067)	46 (0-79)	98 (92-100)
Aggressiveness	4.3% (28/651)	2.3 (0.96-5.6; p=0.063)	44 (0-82)	98 (90-100)
Not being herself/himself	2.3% (15/651)	4.4 (1.5-13; p=0.0080)	79 (0-98)	90 (50-100)
Dysuria	1.8% (12/651)	1.6 (0.47-5.7; p=0.44)	46 (0-90)	98 (82-100)
Urgency	5.5% (36/651)	1.3 (0.58-2.9; p=0.52)	17 (0-70)	99 (95-100)

¹ *Escherichia coli* (*E. coli*) was found in the urine in 143 of 651 urine samples.

² Appearance or increase of symptom or sign within the last three months in the studied population.

³ Statistical correlation between *E. coli* in urine and presence of symptom. Odds ratios are adjusted for age and gender.

⁴ Clinical usefulness evaluated by etiologic predictive value (EPV). EPV is the probability for a positive/negative urine culture to rule in, or rule out that a symptom is associated with a bacterial finding.

Table 8. Odds ratios and probabilities that findings of any bacteria in the urine¹ are associated with symptoms (Paper II)

	Prevalence of symptom ²	Statistical correlation ³	Clinical relevance ⁴	
		Adjusted odds ratio (95% CI; p-value)	Positive EPV % (95% CI)	Negative EPV % (95% CI)
Restlessness	9.1% (59/651)	1.3 (0.76-2.4; p=0.31)	31 (0-71)	98 (91-100)
Fatigue	12% (80/651)	1.7 (1.0-2.7; p=0.046)	40 (0-72)	97 (91-100)
Confusion	7.5% (49/651)	1.9 (1.0-3.5; p=0.044)	48 (0-80)	96 (86-100)
Aggressiveness	4.3% (28/651)	1.7 (0.77-3.9; p=0.19)	31 (0-78)	98 (87-100)
Not being herself/himself	2.3% (15/651)	3.3 (1.1-9.9; p=0.030)	74 (0-99)	88 (17-100)
Dysuria	1.8% (12/651)	1.4 (0.43-4.6; p=0.57)	37 (0-90)	97 (72-100)
Urgency	5.5% (36/651)	1.7 (0.85-3.5; p=0.13)	38 (0-79)	97 (87-100)
Fever	0.31% (2/651)	3.2 (0.17-61; p=0.44)	56 (0-100)	94 (0-100)

¹ Any bacteria was found in the urine in 207 of 651 urine samples.

² Appearance or increase of symptom or sign within the last three months in the studied population. All patients with fever acquired this within the last week.

³ Statistical correlation between any bacteria in urine and presence of symptom. Odds ratios are adjusted for age and gender.

⁴ Clinical usefulness evaluated by etiologic predictive value (EPV). EPV is the probability for a positive/negative urine culture to rule in, or rule out that a symptom is associated with a bacterial finding.

Paper III

A positive urine culture was only significant in the model predicting confusion, OR 0.15 (0.033-0.68; p=0.014). However, it is important to note that the odds ratio is <1, i.e. positive urine cultures are less common among residents with confusion (Paper III, Table 3). As urine IL-6 ≥ 5 ng/L was also a significant predictor in this regression model for confusion, another regression was made where urine culture and urine IL-6 ≥ 5 ng/L were replaced by a combined dichotomous variable being positive if both IL-6 ≥ 5 ng/L and the urine culture was positive at the same time, or otherwise negative. This combined variable was however not a significant predictor of confusion.

4.5 Urine IL-6 and creatinine (Paper III)

Concentrations of IL-6 were analysed in urine specimens from 409/421 residents in 2012. In 12/421 residents, urine samples for IL-6 analyses were accidentally lost, or there was not enough urine for both culture and IL-6 analysis.

Concentration of IL-6 in the urine had a mean of 3.4 ng/L (SD 5.9) and a median of 1.6 ng/L (interquartile range 0.7-4.1, range 0.20-62).

Concentration of creatinine in the urine had a mean of 7.4 mmol/L (SD 4.0). Creatinine adjusted concentration of IL-6 in the urine had a mean of 0.59 ng/mmol creatinine (SD 1.2) and a median of 0.23 ng/mmol creatinine (interquartile range 0.11-0.55, range 0.019-12). Pearson's correlation coefficient between unadjusted urine IL-6 concentrations and creatinine adjusted IL-6 concentrations was 0.86 ($p < 10^{-6}$).

Urine IL-6 concentrations were ≥ 5.0 ng/L in 18% (75/409) of residents and creatinine adjusted IL-6 concentrations were ≥ 0.75 ng/mmol in 18% (75/409) of residents.

IL-6 concentrations in the urine divided by positive and negative urine cultures

Concentrations of IL-6 in the urine was higher ($p = 0.000004$) among residents with significant growth of bacteria in the urine; the mean IL-6 concentration was 5.1 ng/L (SD 8.7) and the median IL-6 concentration was 2.5 ng/L (interquartile range 1.0-5.7), compared to residents with negative urine cultures, where the mean IL-6 concentration was 2.6 ng/L (SD 3.6) and the median IL-6 concentration was 1.3 ng/L (interquartile range 0.6-2.8). The same applies for creatinine adjusted IL-6 concentrations ($p < 10^{-6}$).

Similarly residents with positive urine cultures were more likely to have urine IL-6 ≥ 5.0 ng/L ($p = 0.000053$) and creatinine adjusted IL-6 ≥ 0.75 ng/mmol ($p = 0.000001$) compared to those with negative urine cultures.

4.6 IL-6 and dipstick urinalysis in residents with bacteriuria (Paper III)

In residents exclusively with bacteriuria there were no significant differences in concentrations of urine IL-6 when comparing those with or without a new or increased symptom; fatigue ($p = 0.24$), restlessness ($p = 0.40$), confusion ($p = 0.38$), aggressiveness ($p = 0.66$), loss of appetite ($p = 0.27$), frequent falls ($p = 0.15$), not being herself/himself ($p = 0.90$), having any of the nonspecific symptoms ($p = 0.69$), dysuria ($p = 0.13$) and urinary urgency ($p = 0.82$).

In residents exclusively having bacteriuria there were no significant differences in the proportion of leukocyte esterase dipsticks $\geq 3+$ when comparing those with or without new or increased symptoms; fatigue ($p = 0.39$), restlessness ($p = 1.0$), confusion ($p = 1.0$), aggressiveness ($p = 0.62$), loss of appetite ($p = 1.0$), frequent falls ($p = 0.60$), not being herself/himself ($p = 1.0$), having any of the nonspecific symptoms ($p = 0.68$), dysuria ($p = 0.46$) and

urinary urgency ($p=0.34$). Similarly there were no significant differences in proportion of positive nitrite dipsticks when comparing those with or without new or increased symptoms.

All patients with urinary frequency had negative urine culture.

4.7 Antimicrobial resistance (Paper IV)

4.7.1 Resistance rates in *E. coli*

The average resistance rates for all tested antibiotics were similar between 2003 and 2012, however there was a non-significant trend ($p=0.090$) towards higher resistance rates for cefadroxil in 2012, but still at the low level of 2.6% for all *E. coli* isolates (Table 9). It was not possible to compare resistance rates for quinolones between 2003 and 2012 as nalidixic acid was no longer used as a screening disk for quinolone resistance in 2012.

Table 9. Resistance rates in *Escherichia coli*¹, voided urine specimens (Paper IV)

	2003 (n=143) ²	2012 (n=117) ²	P-value ³
Mecillinam	4.2% (6)	7.7% (9)	0.23
Ampicillin	18% (26)	21% (25)	0.52
Cefadroxil	0.0% (0)	2.6% (3)	0.090
Trimethoprim	13% (18)	12% (14)	0.88
Nitrofurantoin	0.70% (1)	0.85% (1)	1.0
Nalidixic acid	12% (17)	Not tested	---
Ciprofloxacin	Not tested	3.4% (4)	---
Classic ESBL ⁴		1.7% (2)	
AmpC ⁵		0.85% (1)	

¹ Sparse and significant growth of *Escherichia coli*.

² Proportion of resistant *Escherichia coli* % (number).

³ Pearson chi-square and, when appropriate, Fisher's exact test.

⁴ *E. coli* producing extended spectrum beta-lactamase enzymes.

⁵ *E. coli* with plasmid mediated AmpC production.

In 2012, there were two isolates of *E. coli* producing extended spectrum beta-lactamase (classic ESBL), and one isolate with plasmid mediated AmpC production. No carbapenemases were detected. No other ESBL-producing Enterobacteriaceae were found.

In 2003, no ESBL-producing Enterobacteriaceae were found.

In urine specimens obtained from urinary catheters in 2012, resistance rates in *E. coli* were; for ampicillin 46% (6/13), trimethoprim 15% (2/13), mecillinam 0% (0/13), ciprofloxacin 15% (2/13), cefadroxil 7.7% (1/13) and nitrofurantoin 0% (0/13). There was a trend towards higher resistance rates in *E. coli* in urine specimens from catheters compared to voided urine for ampicillin ($p=0.079$) and ciprofloxacin ($p=0.11$).

4.7.2 Predictors of UTI antibiotic resistance in *E. coli*

Antibiotic courses during the previous month increased the risk for resistance in *E. coli*, adjusted for age and gender; for mecillinam with odds ratio 7.1 (2.4-21; $p=0.00049$), ampicillin OR 5.2 (2.4-11; $p=0.00036$), nalidixic acid OR 4.6 (1.4-16; $p=0.014$) and trimethoprim OR 3.9 (1.6-9.2; $p=0.0023$). Predictors for antibiotic resistance in voided urine specimens are presented in Table 10.

In 2012 any overnight admission to hospital was registered. Of those in 2012 with *E. coli* in voided urine samples, 23/117 (20%) had been hospitalized during the previous six months.

Cramer's V between hospital admissions and any antibiotic treatment was calculated, and there was no correlation between "any hospitalization during the six last months" and "any antibiotic treatment during the six last months", 0.090 ($p=0.33$).

To evaluate the impact of hospitalization on antimicrobial resistance rates, logistic regressions were performed, and odds ratios regarding the presence of antibiotic resistance, adjusted for age, gender and any antibiotic treatment during the previous six months, was for ciprofloxacin 13 (1.1-153; $p=0.040$), ampicillin 5.2 (1.8-15; $p=0.0021$), cefadroxil 8.6 (0.71-103; $p=0.091$), trimethoprim 2.4 (0.70-8.5; $p=0.16$), mecillinam 2.1(0.48-9.5; $p=0.32$), nitrofurantoin 0.0 (0.0- ∞ ; $p=1.0$) and any antimicrobial tested for 4.4 (1.6-12; $p=0.0033$).

Table 10. Predictors of UTI antibiotic resistance in *Escherichia coli*¹ in voided urine specimens (Paper IV)

	Unadjusted odds ratios (95% CI; p-value)	Adjusted odds ratios (95% CI; p-value)
Mecillinam		
Antibiotics last month ²	7.2 (2.4-21; p=0.00040)	7.1 (2.4-21; p=0.00049)
Age	1.0 (0.94-1.1; p=0.65)	1.0 (0.94-1.1; p=0.62)
Gender ³	<10 ⁻⁶ (0-∞; p=1.0)	<10 ⁻⁶ (0-∞; p=1.0)
Ampicillin		
Antibiotics last month ²	5.2 (2.4-11; p=0.000030)	5.2 (2.4-11; p=0.000036)
Age	1.0 (0.98-1.1; p=0.40)	1.0 (0.97-1.1; p=0.42)
Gender ³	0.26 (0.033-2.0; p=0.20)	0.32 (0.040-2.6; p=0.28)
Cefadroxil		
Antibiotics last month ²	<10 ⁻⁶ (0-∞; p=1.0)	<10 ⁻⁶ (0-∞; p=1.0)
Age	1.1 (0.91-1.4; p=0.28)	1.1 (0.91-1.4; p=0.30)
Gender ³	<10 ⁻⁶ (0-∞; p=1.0)	<10 ⁻⁶ (0-∞; p=1.0)
Trimethoprim		
Antibiotics last month ²	4.0 (1.7-9.4; p=0.0018)	3.9 (1.6-9.2; p=0.0023)
Age	1.0 (0.96-1.1; p=0.60)	1.0 (0.96-1.1; p=0.65)
Gender ³	<10 ⁻⁶ (0-∞; p=1.0)	<10 ⁻⁶ (0-∞; p=1.0)
Nitrofurantoin		
Antibiotics last month ²	1.0x10 ⁸ (0-∞; p=1.0)	1.1x10 ⁸ (0-∞; p=0.99)
Age	1.2 (0.90-1.6; p=0.22)	1.3 (0.88-1.8; p=0.21)
Gender ³	<10 ⁻⁶ (0-∞; p=1.0)	1.2x10 ⁻⁵ (0-∞; p=1.0)
Nalidixic acid⁴ 2003		
Antibiotics last month ²	4.3 (1.4-13; p=0.013)	4.6 (1.4-16; p=0.014)
Age	0.92 (0.87-0.98; p=0.014)	0.92 (0.86-0.99; p=0.018)
Gender ³	<10 ⁻⁶ (0-∞; p=1.0)	<10 ⁻⁶ (0-∞; p=1.0)
Ciprofloxacin⁵ 2012		
Antibiotics last month ²	3.1 (0.30-32; p=0.35)	3.6 (0.32-40; p=0.31)
Age	1.1 (0.91-1.3; p=0.38)	1.1 (0.90-1.3; p=0.36)
Gender ³	<10 ⁻⁶ (0-∞; p=1.0)	<10 ⁻⁶ (0-∞; p=1.0)

¹ N= 255 (22 sparse growth and 233 significant growth), voided urine specimens in 2003 and 2012 from patients with known antibiotic treatment history.

² Any ongoing (n=8) or previous antibiotic treatment during the last month (n=25).
Reference category: no antibiotics last month.

³ Reference category: female.

⁴ Only includes residents from 2003 since nalidixic acid only was tested in 2003 (n=138).

⁵ Only includes residents from 2012 since ciprofloxacin only was tested in 2012 (n=117).

4.7.3 Resistance rates in *Klebsiella* spp.

The resistance rates among isolated *Klebsiella* spp. in voided urine in 2003 were as follows; for ampicillin 96% (22/23), nitrofurantoin 96% (22/23), mecillinam 13% (3/23), nalidixic acid 9.0% (2/23), trimethoprim 0% (0/23) and cefadroxil 0% (0/23).

The resistance rates among isolated *Klebsiella* spp. in voided urine in 2012 were; for ampicillin 91% (10/11), nitrofurantoin 91% (10/11), mecillinam 0% (0/11), ciprofloxacin 0% (0/11), trimethoprim 27% (3/11) and cefadroxil 0% (0/11). Similar rates were seen in 10 *Klebsiella* isolates from indwelling urinary catheters.

4.8 Dipstick urinalysis

In 2003 (Paper I), visual readings of dipsticks were performed for nitrite in 650/651 residents and for leukocyte esterase in 630/651 residents. Analyser readings of nitrite and leukocyte esterase were performed in 643/651 and 642/651 residents respectively.

In Table 11, the proportion of positive urine dipsticks (visually read in 2003) are presented for; all residents irrespective of outcome of urine cultures, among residents with bacteriuria and finally among residents without bacteriuria.

Table 11. Proportion of positive urine dipsticks in 2003 (Paper I)

	All residents	Residents with bacteriuria ¹	Residents without bacteriuria ¹
LE ² dipstick \geq 1	47% (294/630)	69% (141/205)	36% (153/425)
Positive nitrite ³	24% (154/650)	57% (117/207)	8.4% (37/443)
Positive nitrite <i>and</i> or LE dipstick \geq 1	53% (333/631)	82% (168/205)	39% (165/426)

¹Significant and spares growth of bacteria in voided urine specimens.

²Leukocyte esterase dipstick visually read in 630/651 residents.

³Nitrite dipstick visually read in 650/651 residents.

In 2012 (Paper III), urine dipsticks were analysed visually for nitrite and leukocyte esterase in 408/421 residents. Dipstick urinalysis were not performed in 13/421 residents, mostly due to insufficient urine volume. Among all residents in 2012, regardless of bacteriuria or not, 26% (106/408) of nitrite dipsticks were positive and 22% (90/408) of leukocyte esterase dipsticks were \geq 3+.

In Table 12, the proportion of positive urine dipsticks (visually read in 2012) are presented; among all residents irrespective of outcome of urine cultures, among residents with bacteriuria and finally among residents without bacteriuria.

Table 12. Proportion of positive urine dipsticks in 2012 (Paper III)

	All residents	Residents with bacteriuria ¹	Residents without bacteriuria ¹
LE ² dipstick ≥ 1	43% (175/408)	69% (91/132)	30% (84/276)
Positive nitrite ³	26% (106/408)	64% (84/132)	8.0% (22/276)
Positive nitrite <i>and</i> or LE dipstick ≥ 1	50% (202/408)	86% (114/132)	32% (88/276)

¹Significant growth of bacteria in voided urine specimens.

²Leukocyte esterase dipstick visually read in 408/421 residents.

³Nitrite dipstick visually read in 408/421 residents.

4.8.1 Agreement between visual and analyser readings (Paper I)

Visual and analyser readings had, for nitrite, good agreement with a kappa coefficient 0.92 (95% confidence interval 0.88-0.95, SE for kappa 0.019). However, the agreement for leukocyte esterase was lower with kappa coefficient 0.54 (95% confidence interval 0.49-0.60, SE for kappa 0.027).

4.8.2 Sensitivity and specificity (Paper I)

Sensitivity and specificity of urine dipstick analyses regarding leukocyte esterase and nitrite are presented in Tables 13 and 14. Sensitivity and specificity for combinations of the dipsticks are presented in Tables 15 and 16.

4.8.3 NPV and PPV (Paper I)

If a single leukocyte esterase dipstick was negative then the high NPV showed it unlikely for the urine culture to be positive for *E. coli*, *E. faecalis* and *Klebsiella* spp. (Table 13). However, presence of “any bacteria” could not be excluded (Table 13). If a single leukocyte esterase dipstick was positive it could not sufficiently predict bacteriuria (Table 13).

Where a single nitrite dipstick was negative the high NPV showed it unlikely that the culture was positive for *E. coli*, *E. faecalis* and *Klebsiella* spp. respectively (Table 14). However, presence of “any bacteria” could not be excluded (Table 14). If a single nitrite dipstick was positive it could not sufficiently predict bacteriuria (Table 14). A single negative nitrite dipstick was as good as a single negative leukocyte esterase dipstick in excluding presence of *E. coli* (Tables 13 and 14). Although a single positive nitrite dipstick could not sufficiently predict *E. coli* in the urine, it was better than a single leukocyte esterase dipstick. Raising the cut-off point in a single

leukocyte esterase dipstick decreased its ability to exclude *E. coli* without making PPV for *E. coli* acceptable (Table 13). Thus, if a single dipstick was to exclude or predict the presence of *E. coli*, then the nitrite dipstick performed better than the leukocyte esterase dipstick. The accuracy of excluding or predicting *E. faecalis* and *Klebsiella* spp. did not differ between a single leukocyte esterase dipstick or a single nitrite dipstick (Tables 13 and 14).

Combining the two dipsticks, so that presence of both leukocyte esterase and nitrite were considered a positive test and all other test outcomes as negative, altered test characteristics slightly compared to using only one of the dipsticks (Table 15). NPV for predicting absence of *E. coli* was lower compared to using only one of the dipsticks while PPV for predicting presence of *E. coli* increased only marginally.

Combining the two dipsticks so that presence of leukocyte esterase and/or nitrite were considered positive and all other test outcomes as negative also altered test characteristics compared to using only one of the dipsticks (Table 16). The NPV for predicting absence of a specified potentially pathogenic bacteria or predicting “any bacteria” was high enough to rule out bacteriuria.

4.8.4 Predicting bacteriuria, adjusted OR (Paper I)

The association between dipstick findings and urine culture was further evaluated by logistic regression to consider age or gender dependent effects. A visually read leukocyte esterase dipstick >0 added information to the question of whether *E. coli* and “any bacteria” were present in the urine (Table 17). For *E. faecalis* and *Klebsiella* spp. a leukocyte esterase dipstick added information in some colour blocks but not in others (Table 17).

A positive nitrite dipstick, visually or analyser read, added information to the question of whether *E. coli*, *Klebsiella* spp. or “any bacteria” was present in the urine (Table 17). For *E. faecalis* a nitrite dipstick added no statistically significant information (Table 17).

4.8.5 Association between dipstick findings, symptoms and urine cultures (Paper III)

Leukocyte esterase dipsticks $\geq 3+$ were more common ($p < 10^{-6}$) among residents with significant growth of bacteria in the urine; 46% (61/132) versus 11% (29/276) in residents with negative urine cultures. Positive nitrite dipsticks were more common ($p < 10^{-6}$) among residents with positive urine cultures; 64% (84/132) versus 8.0% (22/276) in residents with negative urine cultures.

Table 13. Test characteristics of a single leukocyte esterase dipstick compared to urine culture.

	<i>Escherichia coli</i> ^a		<i>Enterococcus faecalis</i> ^b		<i>Klebsiella</i> species ^c		Any bacteria ^d		
	Visual reading ^e	Analysed reading ^f	Visual reading ^e	Analysed reading ^f	Visual reading ^e	Analysed reading ^f	Visual reading ^e	Analysed reading ^f	
Sensitivity	>0	72% (64-79)	81% (75-88)	71% (49-92)	82% (64-100)	68% (50-86)	71% (53-89)	69% (62-75)	78% (73-84)
	>1	59% (51-67)	62% (54-70)	59% (35-82)	65% (42-87)	60% (41-79)	58% (39-78)	57% (50-64)	61% (54-67)
	>2	37% (29-45)	38% (30-46)	47% (23-71)	41% (18-65)	52% (32-72)	46% (26-66)	37% (30-44)	38% (31-44)
	>3	11% (6.1-17)	17% (11-24)	18% (0.0-36)	29% (7.8-51)	12% (0.0-25)	25% (7.7-42)	11% (6.5-15)	18% (13-23)
Specificity	>0	61% (56-65)	52% (48-57)	54% (50-58)	46% (42-50)	54% (50-58)	45% (42-49)	64% (59-69)	56% (51-60)
	>1	74% (70-78)	73% (69-76)	67% (64-71)	66% (62-69)	68% (64-71)	66% (62-69)	78% (74-82)	77% (73-81)
	>2	84% (80-87)	83% (80-86)	80% (77-83)	79% (76-82)	80% (77-84)	79% (76-82)	87% (84-90)	86% (83-89)
	>3	96% (94-98)	92% (89-94)	94% (93-96)	90% (88-93)	94% (93-96)	90% (88-93)	96% (95-98)	93% (91-96)
PPV	>0	34% (29-40)	33% (28-38)	4.1% (1.8-6.3)	4.0% (1.9-6.0)	5.8% (3.1-8.5)	4.8% (2.6-7.0)	48% (42-54)	45% (40-51)
	>1	40% (33-46)	39% (33-46)	4.8% (1.9-7.6)	4.9% (2.1-7.7)	7.1% (3.7-11)	6.2% (3.1-9.3)	56% (49-62)	55% (49-62)
	>2	39% (31-48)	40% (31-48)	6.1% (2.0-10)	5.0% (1.4-8.7)	9.9% (4.8-15)	7.9% (3.4-12)	58% (49-66)	56% (48-64)
	>3	43% (27-59)	38% (26-50)	8.1% (0.0-17)	7.6% (1.2-14)	8.1% (0.0-17)	9.1% (2.2-16)	59% (44-75)	56% (44-68)
NPV	>0	88% (85-92)	91% (87-94)	99% (97-100)	99% (98-100)	98% (96-99)	98% (96-99)	81% (77-85)	84% (80-89)
	>1	86% (83-89)	87% (84-90)	98% (97-100)	99% (97-100)	98% (96-99)	98% (96-99)	79% (75-83)	81% (77-84)
	>2	82% (79-85)	83% (79-86)	98% (97-99)	98% (97-99)	98% (96-99)	97% (96-99)	74% (70-78)	75% (71-78)
	>3	79% (76-82)	80% (76-83)	98% (96-99)	98% (97-99)	96% (95-98)	97% (95-98)	69% (65-73)	71% (67-74)

^a 143 of 651 urine cultures showed growth of *Escherichia coli*.

^b 17 of 651 urine cultures showed growth of *Enterococcus faecalis*.

^c 25 of 651 urine cultures showed growth of *Klebsiella* spp.

^d 207 of 651 urine cultures showed growth of any bacteria. Any bacteria may be *E. coli*, *E. faecalis*, *Klebsiella* spp., *E. faecium*, *Enterobacter* spp., *coagulase-negative staphylococci*, *alfa-hemolytic streptococci*, *beta-hemolytic streptococci*, *Proteus mirabilis*, *P. vulgaris*, *Group B Streptococci* and *Pseudomonas aeruginosa*.

^e Number of visual readings: 630

^f Number of analyser readings: 642

Table 14. Test characteristics of a single nitrite dipstick compared to urine culture.

	<i>Escherichia coli</i> ^a		<i>Enterococcus faecalis</i> ^b		<i>Klebsiella</i> species ^c		Any bacteria ^d	
	Visual reading ^e	Analyser reading ^f	Visual reading ^e	Analyser reading ^f	Visual reading ^e	Analyser reading ^f	Visual reading ^e	Analyser reading ^f
Sensitivity	64% (56-72)	62% (54-70)	29% (7.8-51)	29% (7.8-51)	48% (28-68)	50% (30-70)	57% (50-63)	56% (49-62)
Specificity	88% (85-90)	86% (83-89)	76% (73-80)	76% (73-79)	77% (74-81)	77% (73-80)	92% (89-94)	90% (88-93)
PPV	59% (51-67)	56% (49-64)	3.3% (0.45-6.1)	3.2% (0.44-6.0)	7.8% (3.6-12)	7.7% (3.5-12)	76% (69-83)	73% (66-80)
NPV	90% (87-92)	89% (86-92)	98% (96-99)	98% (96-99)	97% (96-99)	98% (96-99)	82% (78-85)	81% (78-85)

^a 143 of 651 urine cultures showed growth of *Escherichia coli*.

^b 17 of 651 urine cultures showed growth of *Enterococcus faecalis*.

^c 25 of 651 urine cultures showed growth of *Klebsiella* spp.

^d 207 of 651 urine cultures showed growth of any bacteria. Any bacteria may be *E. coli*, *E. faecalis*, *Klebsiella* spp., *E. faecium*, *Enterobacter* spp., *coagulase-negative*

staphylococci, *alfa-hemolytic streptococci*, *beta-hemolytic streptococci*, *Proteus mirabilis*, *P. vulgaris*, *Group B Streptococci* and *Pseudomonas aeruginosa*.

^e Number of visual readings: 650

^f Number of analyser readings: 643

Table 15. Test characteristics of a positive leukocyte esterase and a positive nitrite dipstick compared to urine culture.

	<i>Escherichia coli</i> ^a		<i>Enterococcus faecalis</i> ^b		<i>Klebsiella</i> species ^c		Any bacteria ^d	
	Visual reading ^e	Analyser reading ^f	Visual reading ^e	Analyser reading ^f	Visual reading ^e	Analyser reading ^f	Visual reading ^e	Analyser reading ^f
Sensitivity	>0	50% (42-59)	18% (0.0-36)	24% (3.4-44)	36% (17-55)	42% (22-61)	44% (37-51)	48% (41-55)
	>1	42% (34-50)	18% (0.0-36)	18% (0.0-36)	32% (14-50)	33% (14-52)	37% (30-44)	40% (33-46)
	>2	25% (18-32)	27% (19-34)	12% (0.0-27)	29% (11-47)	29% (11-47)	23% (18-29)	25% (19-31)
	>3	8.5% (3.9-13)	12% (6.6-17)	0.0% (0.0-0.0)	5.9% (0.0-17)	8.0% (0.0-19)	7.3% (3.8-11)	11% (6.9-16)
Specificity	>0	91% (88-94)	82% (79-85)	80% (77-83)	82% (79-86)	80% (77-84)	94% (92-96)	93% (90-95)
	>1	94% (92-96)	86% (83-89)	85% (82-87)	86% (84-89)	85% (82-88)	97% (95-98)	96% (94-98)
	>2	95% (93-97)	91% (88-93)	90% (88-93)	91% (89-93)	91% (89-93)	97% (96-99)	98% (96-99)
	>3	99% (98-100)	98% (96-99)	97% (96-98)	96% (94-97)	97% (96-99)	99% (98-100)	99% (98-100)
PPV	>0	62% (53-71)	2.6% (0.0-5.5)	3.1% (0.1-6.1)	7.8% (2.9-13)	7.7% (3.1-12)	78% (71-86)	76% (69-83)
	>1	66% (56-75)	3.3% (0.0-7.0)	3.0% (0.0-6.4)	8.9% (3.0-15)	8.1% (2.7-13)	84% (77-92)	82% (74-89)
	>2	58% (46-71)	62% (50-74)	3.3% (0.0-7.9)	3.3% (0.0-7.8)	12% (3.5-20)	80% (70-90)	84% (74-93)
	>3	63% (41-85)	61% (43-79)	0.0% (0.0-0.0)	3.6% (0.0-10)	11% (0.0-24)	79% (61-97)	82% (68-96)
NPV	>0	86% (83-89)	87% (84-90)	97% (96-99)	97% (96-99)	97% (96-99)	78% (74-81)	79% (76-83)
	>1	85% (82-88)	86% (83-88)	97% (96-99)	97% (96-99)	97% (96-98)	76% (73-80)	77% (73-81)
	>2	81% (78-85)	82% (79-85)	97% (96-99)	97% (96-99)	97% (95-98)	72% (69-76)	73% (70-77)
	>3	79% (76-82)	79% (76-83)	97% (96-99)	97% (96-99)	96% (95-98)	69% (65-73)	70% (66-74)

^a 143 of 651 urine cultures showed growth of *Escherichia coli*.^b 17 of 651 urine cultures showed growth of *Enterococcus faecalis*.^c 25 of 651 urine cultures showed growth of *Klebsiella* spp.^d 207 of 651 urine cultures showed growth of any bacteria. Any bacteria may be *E. coli*, *E. faecalis*, *Klebsiella* spp., *E. faecium*, *Enterobacter* spp., *coagulase-negative staphylococci*, *alpha-hemolytic streptococci*, *beta-hemolytic streptococci*, *Proteus mirabilis*, *P. vulgaris*, *Group B Streptococci* and *Pseudomonas aeruginosa*.^e Number of visual readings: 630^f Number of analyser readings: 637

Table 16. Test characteristics of a positive leukocyte esterase and/or a positive nitrite dipstick compared to urine culture.

	<i>Escherichia coli</i> ^a		<i>Enterococcus faecalis</i> ^b		<i>Klebsiella species</i> ^c		Any bacteria ^d		
	Visual reading ^e	Analysar reading ^f	Visual reading ^e	Analysar reading ^f	Visual reading ^e	Analysar reading ^f	Visual reading ^e	Analysar reading ^f	
Sensitivity	>0	86% (80-92)	88% (83-93)	82% (64-100)	88% (73-100)	80% (64-96)	79% (63-95)	82% (77-87)	85% (81-90)
	>1	82% (75-88)	80% (73-86)	71% (49-92)	76% (56-97)	76% (59-93)	75% (58-92)	77% (71-83)	77% (71-83)
	>2	77% (70-84)	74% (67-81)	65% (42-87)	59% (35-82)	72% (54-86)	67% (48-86)	71% (65-77)	69% (62-75)
	>3	67% (60-75)	68% (60-75)	47% (23-71)	53% (29-77)	52% (32-72)	63% (43-82)	60% (54-67)	62% (56-69)
Specificity	>0	57% (52-61)	49% (44-53)	48% (44-52)	41% (38-45)	48% (44-52)	41% (38-45)	61% (57-66)	53% (48-58)
	>1	68% (64-72)	66% (62-70)	57% (54-61)	57% (53-61)	58% (54-62)	57% (53-61)	73% (69-77)	71% (67-76)
	>2	76% (72-80)	74% (70-78)	65% (61-69)	64% (60-68)	66% (62-70)	65% (61-68)	81% (77-85)	79% (75-83)
	>3	84% (81-88)	80% (77-84)	73% (70-77)	70% (67-74)	74% (70-77)	71% (67-74)	89% (86-92)	85% (81-88)
PPV	>0	36% (31-42)	33% (28-38)	4.2% (2.1-6.4)	4.0% (2.0-5.9)	6.0% (3.5-8.6)	5.0% (2.8-7.2)	51% (45-56)	46% (41-51)
	>1	42% (36-48)	40% (34-46)	4.4% (2.0-6.8)	4.6% (2.2-7.1)	7.0% (3.9-10)	6.4% (3.5-9.2)	58% (52-64)	56% (50-62)
	>2	48% (41-55)	45% (39-51)	4.9% (2.1-7.7)	4.3% (1.7-6.9)	8.0% (4.5-12)	6.9% (3.6-10)	64% (58-71)	61% (54-67)
	>3	56% (48-63)	49% (42-57)	4.7% (1.5-7.8)	4.6% (1.7-7.6)	7.6% (3.6-12)	7.7% (4.0-11)	73% (66-79)	66% (59-73)
NPV	>0	93% (90-96)	93% (90-96)	99% (98-100)	99% (98-100)	98% (97-100)	98% (96-100)	88% (84-91)	88% (85-92)
	>1	93% (90-95)	92% (89-95)	99% (97-100)	99% (98-100)	98% (97-100)	98% (97-100)	87% (83-90)	87% (83-90)
	>2	92% (89-95)	91% (88-94)	99% (97-100)	98% (97-100)	98% (97-100)	98% (97-99)	85% (82-89)	84% (81-88)
	>3	90% (87-93)	90% (87-92)	98% (97-99)	98% (97-99)	97% (96-99)	98% (97-99)	82% (79-86)	83% (79-86)

^a 143 of 651 urine cultures showed growth of *Escherichia coli*.

^b 17 of 651 urine cultures showed growth of *Enterococcus faecalis*.

^c 25 of 651 urine cultures showed growth of *Klebsiella* spp.

^d 207 of 651 urine cultures showed growth of any bacteria. Any bacteria may be *E. coli*, *E. faecalis*, *Klebsiella* spp., *E. faecium*, *Enterobacter* spp., *coagulase-negative staphylococci*, *alfa-hemolytic streptococci*, *beta-hemolytic streptococci*, *Proteus mirabilis*, *P. vulgaris*, *Group B Streptococci* and *Pseudomonas aeruginosa*.

^e Number of visual readings: 630

^f Number of analyser readings: 637

Table 17. Odds ratio for a positive dipstick to predict presence of bacteriuria when considering age and sex.

	<i>Escherichia coli</i> ^a		<i>Enterococcus faecalis</i> ^b		<i>Klebsiella</i> species ^c		Any bacteria ^d	
	Visual reading ^e	Analysed reading ^f	Visual reading ^e	Analysed reading ^f	Visual reading ^e	Analysed reading ^f	Visual reading ^e	Analysed reading ^f
Leukocyte esterase	>0 3.3 (2.1-4.9) --- <0.001 >1 3.6 (2.4-5.4) --- <0.001 >2 2.7 (1.8-4.2) --- <0.001 >3 2.8 (1.4-5.7) --- 0.0043	3.7 (2.3-5.9) --- <0.001 3.9 (2.6-5.9) --- <0.001 2.7 (1.8-4.2) --- <0.001 2.0 (1.2-3.5) --- 0.014	3.3 (1.1-9.8) --- 0.033 3.3 (1.2-9.2) --- 0.020 3.7 (1.4-10) --- 0.0094 3.7 (1.0-14) --- 0.050	4.7 (1.3-17) --- 0.020 3.7 (1.3-10) --- 0.012 2.8 (1.0-7.6) --- 0.048 3.9 (1.3-12) --- 0.016	2.1 (0.90-5.1) --- 0.087 2.8 (1.2-6.4) --- 0.014 4.1 (1.8-9.3) --- <0.001 2.2 (0.63-7.8) --- 0.22	1.6 (0.65-4.1) --- 0.30 2.4 (1.0-5.6) --- 0.038 2.9 (1.3-6.8) --- 0.011 2.9 (1.1-7.7) --- 0.034	3.4 (2.4-4.9) --- <0.001 4.3 (3.0-6.2) --- <0.001 3.7 (2.4-5.5) --- <0.001 3.3 (1.6-6.6) --- <0.001	3.8 (2.5-5.6) --- <0.001 4.7 (3.3-6.8) --- <0.001 3.4 (2.3-5.1) --- <0.001 2.7 (1.6-4.6) --- <0.001
Nitrite	Pos 12 (7.5-18) --- <0.001	9.8 (6.3-15) --- <0.001	1.4 (0.49-4.2) --- 0.52	1.4 (0.48-4.1) --- 0.54	2.8 (1.2-6.2) --- 0.015	2.9 (1.3-6.7) --- 0.012	14 (6.7-21) --- <0.001	11 (7.3-17) --- <0.001

^a 143 of 651 urine cultures showed growth of *Escherichia coli*.

^b 17 of 651 urine cultures showed growth of *Enterococcus faecalis*.

^c 25 of 651 urine cultures showed growth of *Klebsiella* spp.

^d 207 of 651 urine cultures showed growth of any bacteria. Any bacteria may be *E. coli*, *E. faecalis*, *Klebsiella* spp., *E. faecium*, *Enterobacter* spp., *coagulase-negative staphylococci*, *alpha-hemolytic streptococci*, *beta-hemolytic streptococci*, *Proteus mirabilis*, *P. vulgaris*, *Group B Streptococci* and *Pseudomonas aeruginosa*.

^e Number of visual readings for leukocyte esterase were 630 and 650 for nitrite.

^f Number of analyser readings for leukocyte esterase were 642 and 643 for nitrite.

5 DISCUSSION

5.1 Summary

If there is a reason for testing for bacteriuria, dipstick urinalysis for nitrite and leukocyte esterase can rule out but cannot reliably rule in bacteriuria. There were no clinically relevant differences between visual and analyser readings of urine dipsticks (Paper I).

Recent onset of nonspecific symptoms was common among elderly residents of nursing homes. Residents without nonspecific symptoms had positive urine cultures as often as those with nonspecific symptoms with a duration of up to one month. Nonspecific symptoms among elderly residents of nursing homes are unlikely to be caused by bacteria in the urine. Therefore, dipstick urinalysis and urine cultures are of little or no value in clarifying the aetiology of nonspecific symptoms (Papers II and III).

Residents with positive urine cultures had higher concentrations of IL-6 in the urine. However, among residents with positive urine cultures there were no differences in IL-6 concentrations or dipstick findings between those with or without nonspecific symptoms (Paper III).

There were still comparatively low levels of antimicrobial resistance in urinary pathogens among Swedish nursing home residents with no major changes between 2003 and 2012. Any antibiotic treatment during the last month and hospitalization during the last six months predicted higher resistance rates in *E. coli* (Paper IV).

5.2 Strengths and limitations of the thesis

5.2.1 Urine specimens regardless of symptoms

A major strength of this thesis is that urine specimens were collected from every participating resident capable of providing a urine sample, regardless of the presence of symptoms. Therefore, this thesis can compare residents having symptoms with those without symptoms. This is essential due to the lack of a gold standard to differentiate between patients with a symptomatic UTI and patients whose symptoms are caused by something else, with the bacteria found in the urine being just an ABU. This is further discussed in section 5.3.4.

Another major strength of collecting urine specimens from all residents able to provide a urine sample is the possibility of describing the native antimicrobial resistance in nursing homes for the elderly. Previously, most studies of antimicrobial resistance in uropathogens among residents of nursing homes compiled antimicrobial resistance in urine cultures taken in the event of clinical suspicion of UTI. Those with UTI symptoms and possible underlying diseases in the urinary tract comprised a select patient group assumed to have higher resistance rates. However, clinicians have varying thresholds for suspecting a UTI creating unclarity as to what underlying population these patients represent. The possibility of comparing resistance rates at nursing homes in the same geographical area between 2003 and 2012 is also a strength of this thesis. Furthermore, since this study includes many nursing homes, they can be assumed to be representative of all Swedish nursing homes, especially since nursing homes are similarly organised in all regions of Sweden. The nursing homes were located both in urban and rural areas.

5.2.2 Participation rate

In these studies we obtained urine specimens and study protocols from 55% (651/1187) of the individuals registered at the nursing homes in 2003, and from 53% (480/901) in 2012. This may appear low but is similar to previously published studies screening for bacteriuria in nursing homes for the elderly [5]. The main reason for not participating was substantial urinary incontinence, often combined with dementia. Obtaining a urine specimen from these individuals would have required the use of a urinary catheter, not routine in clinical practice for the elderly at nursing homes, and would, therefore, not have been representative of clinical practice. Furthermore, this would have been dubious from an ethical point of view.

Among registered residents of the nursing homes 8.1% (96/1187) and 25% (222/901) refused participation in 2003 and 2012, respectively. Still this may be considered acceptable when studying an elderly fragile population with a high proportion of residents with dementia as well as the ethical requirement of approval from appointed representatives/relatives.

All individuals living at the nursing homes were asked to participate. Due to ethical considerations, it was not noted whether those who refused participation suffered from dementia or urinary incontinence too severe to be able to provide a urine sample. The same applied to one ward withdrawing during the ongoing study in 2012. Thus, it is assumed that some of the patients excluded, since they refused participation, would not have been eligible for this study anyway. Knowing these numbers would probably have resulted in

less exclusion due to a higher number of residents not meeting the inclusion criteria.

Individuals with indwelling urinary catheters were excluded in Papers I-III since they always become colonized by bacteria, sometimes by different species compared to those without catheters [2, 128].

5.2.3 Evaluation of nonspecific symptoms

Restlessness, fatigue, confusion, aggressiveness, loss of appetite, frequent falls, or in another way not being herself/himself are symptoms not specific for the urinary tract. These nonspecific symptoms are common reasons for suspecting UTI and initiating antibiotic treatment [32, 34, 35, 59, 129], despite clear evidence that these symptoms are associated with a lower UTI.

In this thesis, nonspecific symptoms were not diagnosed by using validated questionnaires or other instruments. Instead, we used the labels used by most nurses for describing patient symptoms when consulting a physician. This is both a strength and a limitation.

From a scientific point of view it might have been better if a psychiatrist had been consulted for the purpose of these studies to evaluate all residents with nonspecific symptoms such as restlessness, confusion and aggressiveness. However, this would never occur in clinical practice when suspecting UTI in nursing homes for the elderly. With such a study design, results would not be generalizable to clinical practice in nursing homes. Since we wanted to study if nonspecific deterioration, reported from attending nurses in clinical practice, was related to positive urine cultures we made the decision to allow the attending nurse to evaluate symptoms. Therefore the results can be considered representative of everyday clinical practice, as nursing home practitioners often rely on the observations of the staff, communicated by telephone, usually not examining the resident before a clinical decision.

The description of not being herself/himself in a way differing from the other nonspecific symptoms, is a vaguer symptom. However, it is frequently used by nursing home personnel to describe a diffuse deterioration among residents and, if associated with bacteriuria, often results in antibiotic treatment [35, 41]. Thus, we wanted to include this frequently used description of deterioration.

In clinical practice, most general practitioners reported accepting the nursing home staff's assessment of the resident and seldom visited a patient in a nursing home for a UTI [41]. Even when a suspected UTI was associated with unstable vital signs, only 29% were seen by a practitioner or transferred to

hospital [130]. In addition, a high proportion of antibiotics were issued in Swedish nursing homes after indirect contact with the physician [14]. The reason for using labels in this thesis, commonly used by nurses, is that the nurse is the key person in identifying symptoms presumed to be associated with UTI. The nurse plays a central role in both the ordering of urine cultures and the prescription of antibiotics through an awareness of changes in residents' status and their consequential communication of this to physicians [35, 41].

The attending nurses were carefully instructed to register presence or absence of symptoms in the study protocol before collecting urine specimens and performing dipstick urinalysis. Thus, the evaluation of symptoms was not influenced by the outcomes of urine tests.

5.2.4 Symptoms from the urinary tract

There was a low prevalence of new or increased dysuria, urgency and urinary frequency described in this thesis compared to studies collecting urine specimens from patients with suspected UTI. This was expected since this thesis consists of cross sectional studies collecting urine specimens from all those able to provide a urine sample at the participating nursing homes, regardless of the presence of symptoms. Due to the low prevalence of urinary tract symptoms, these studies were partially underpowered for specific UTI symptoms. The nonspecific symptoms were the main focus of this thesis and the studies were primarily powered for the more commonly occurring non-specific symptoms.

5.2.5 Ongoing antibiotic treatment

The numbers of nursing home residents with ongoing antibiotic treatment when urine specimens were collected were; in Papers I and II 4.0% (26/651), in Paper III 4.3% (18/421) and in Paper IV 4.8% (23/480). For those individuals on antibiotics affecting urinary pathogens, no growth of urinary pathogens and negative nitrite dipsticks were expected. Due to the relatively low prevalence of ongoing antibiotic treatment this effect was considered low.

5.3 Methodological aspects

5.3.1 Duration of symptoms

Attending physicians are not always consulted immediately when patients develop new or increased nonspecific symptoms in nursing homes. The attending nurses often wait and see if symptoms disappear spontaneously. Thus, there can be some delay before the physician is contacted. As we strove

for these studies to reflect everyday clinical practice we studied symptoms that had existed for some time as well. Depending on duration or increase symptoms were divided into the following groups: less than one week, more than one week but less than one month or more than one month but less than three months. A symptom was not taken into consideration if it had persisted unchanged longer than three months.

Aware of the possibility that different duration intervals could affect results, data was not only analysed in Paper II for symptom duration less than three months, but for each duration interval mentioned separately above. It turned out that in most situations more detailed information for different durations, other than duration of less than three months, was of no great importance.

For the purpose of Paper III we only analysed newly onset or increased symptoms during the last month and still present when the urine specimen was obtained. We abstained from analysing symptom durations of over one month since such long durations are less adequate from a perspective of acute infections.

5.3.2 Dipstick urinalysis

Dipstick urinalysis was performed by non-laboratory personnel in these studies. If the dipstick urinalysis had been performed by laboratory personnel, the results may have differed slightly. On the other hand, these bedside tests are usually performed by non-laboratory personnel in clinical practice at nursing homes. Thus, this study represented ordinary clinical practice.

The kappa coefficient for agreement between visual and analyser readings was lower for leukocyte esterase dipsticks than for nitrite (Paper I). This is logical whereby leukocyte esterase dipsticks have several colour blocks, while nitrite dipsticks have only a binary outcome. The greater the number of outcomes the lower the kappa value.

The NPV for nitrite to predict absence of *E. faecalis* in a urine culture was higher than for *E. coli* despite *E. faecalis* being a poor converter of nitrate to nitrite (Paper I). The most likely explanation being that the prevalence of *E. faecalis* was very low (2.6 %) resulting in a high NPV even if sensitivity and specificity were low.

5.3.3 Urine cultures

Procedures utilizing the presence of symptoms or outcomes of prior dipstick testing to influence setting of cut-off levels for CFU/mL in urine cultures to

label growth as clinically significant may enhance the diagnostic procedure [131-133]. These procedures are common in microbiologic laboratories in Sweden and internationally. This procedure has also been used in previous studies of antimicrobial susceptibility whereby it reflects the clinical situation [81, 134, 135]. Using the routine clinical procedure increases clinical usefulness of the study results. Thus, the present procedure for urine cultures was used without modification in order to be representative of routine clinical practice.

It is important to mention that none of the nonspecific symptoms influenced decisions on cut-off levels for CFU in the urine cultures. Therefore the cut-off levels were not influenced by the main studied symptoms in this thesis. The only symptoms/signs that, for some of the bacteria, lowered the cut-off point were fever, urinary frequency, urgency, dysuria and a positive nitrite dipstick or a leukocyte esterase dipstick >1. Sparse and significant growth are defined in section 3.4.2. In this thesis, few individuals had any specific symptom of UTI, but many had significant growth, which is an important result to consider when evaluating urine specimens in this population.

The label of “significant growth” was used whereby it was unlikely that growth could be explained by contamination of the urine sample, however it did not indicate symptomatic bacteriuria, as it might just as well have represented an ABU.

For the purpose of Paper IV, both significant and sparse growth was defined as a positive urine culture. When including all isolates with sparse or significant growth, the pool of resistant bacteria in the nursing home population was more accurately described in Paper IV.

It was a delicate and difficult question to decide if sparse growth was to be considered or not in Papers I-III. Paper I deals with the question when dipstick urinalysis can rule bacteriuria in or out. For ruling out bacteriuria, the clinician would probably feel more confident if dipstick urinalysis could also rule out sparse growth. Thus we decided to include sparse growth in Paper I. When suspecting a UTI, clinicians would probably also consider treating sparse growth. Thus, we decided to include sparse growth in Paper II. As sparse growth tends to make positive cultures more unspecific we decided not to include sparse growth in Paper III; thus both options were studied in this thesis.

Unfortunately, nalidixic acid was no longer used as a screening disk for quinolone resistance in 2012. It was consequently not possible to compare resistance rates for quinolones between 2003 and 2012.

Only 46% of urine cultures obtained from indwelling urinary catheters were classified as positive in Paper IV. However, there were growths of mixed flora in all but one of the cultures obtained from catheters, classified as negative.

E. coli was by far the most common finding among all isolated species both in 2003 and 2012. However, the proportion of *E. coli* in voided urine was higher in 2012; 80% (117/147) as compared to 69% (143/207) in 2003. This could in part depend on the lower proportion of *E. faecalis* in 2012; 0.68% (1/147) versus 8.2% (17/207) in 2003. Even if these are small numerical differences, we can provide no satisfactory explanation. We have no data indicating more complicating factors within the urinary tract in 2003, since we did not investigate for that.

5.3.4 Lack of gold standard

Changes in mental status are the most common causes for suspecting lower UTI among elderly residents of nursing homes [32, 34, 35]. These nonspecific symptoms can have many causes besides a possible lower UTI [1, 36]. At the same time there is a high prevalence of ABU in this population [2]. Is bacteria in the urine only to be considered an insignificant finding (ABU), or is there a lower UTI causing the new symptom? Unfortunately there are no symptoms, signs, tests or gold standard that can tell us [1]. Thus, it is impossible to know whether a positive urine culture in a patient with a new symptom is related to the new symptom or not.

There are different opinions on the possible association between nonspecific symptoms and lower UTI [1, 18-31]. This scientific and diagnostic uncertainty has its origin in the lack of a gold standard that could determine if bacteriuria has anything to do with a new symptom or if it is just an ABU, i.e. an insignificant finding having nothing to do with the new symptom [1]. This is the main scientific problem to overcome when doing research on symptoms and bacteriuria among elderly residents of nursing homes.

Unfortunately, several studies have used the clinician's judgement, with or without concomitant bacteriuria and/or positive dipstick urinalysis, as a gold standard to judge if it is a symptomatic UTI or not [20, 21, 27, 28]. It is not possible for the clinician to know whether a positive urine culture in a patient with a new symptom is related to the new symptom since the prevalence of asymptomatic bacteriuria is high in this population [1]. Consequently the proportion of positive urine cultures in studies of assumed UTI [21] are usually at par with the proportion of ABU in this population, 25% - 50% for women and 15% - 40% for men [2, 7]. When the proportion of positive urine cultures

are similar in studies of ABU and studies of assumed UTI it is reasonable to conclude that most assumed UTIs in these studies actually were ABU. Studies using the clinician's judgement to decide when a UTI is suspected do not answer the question of what symptoms and signs are appropriate diagnostic criteria for symptomatic UTI in residents with bacteriuria. Instead, it answers the question "What clinical presentations are associated with a positive urine culture?" [29].

A prospective study following individuals over time and recording new symptoms cannot solve the problem of a missing gold standard. It is equally impossible in a prospective study design to know if findings of bacteria in the urine is just a coexisting ABU, or if it represents a symptomatic UTI.

A possible way to handle the lack of a gold standard would be a large randomized controlled trial (RCT), to test if there is any difference in symptom outcome between residents allocated to placebo and UTI antibiotics respectively. However, an RCT in a large population of fragile elderly individuals, many with dementia and no possibility to give statement of consent, would be very difficult to carry out. Another possibility to bypass the problem of a missing gold standard is to use the statistical method of EPV.

5.3.5 Statistical methods

EPV

While results of logistic regressions are of interest on a group level, these results are of limited value to the physician making a clinical decision for a single patient. Predictive values are of greater clinical importance to the physician whereby a positive predictive value could describe the probability of a positive test (findings of bacteriuria) of being associated with a symptom. Ordinary predictive values cannot be calculated in the absence of a gold standard for this situation. However, predictive values in terms of EPV can be calculated, as EPV does not require a gold standard, and statistically considers asymptomatic carriers as well. In Paper II, EPV provides an estimate of the clinical relevance of bacteriuria.

EPV can only be calculated if urine specimens are analysed from both symptomatic and asymptomatic individuals. To calculate EPV for confusion and bacteria in the urine it is necessary to know; how many are confused and have bacteria in the urine; how many are confused but without having bacteria in the urine; how many are not confused but all the same have bacteria in the urine; and how many are not confused and have no bacteria in the urine. When knowing these numbers it is possible to calculate the EPV, which is the

probability for bacteria found in the urine to be associated with confusion. Therefore it is necessary to assess all residents at the nursing homes regardless of whether they have symptoms or not, and irrespective of having bacteriuria or not in order to calculate the EPV. Thus all individuals meeting the inclusion criteria and consenting to participate were assessed.

Logistic regression is a more sensitive statistical method than EPV. It is common that regression shows statistical significance, while a positive EPV does not show that a positive test outcome is clinically relevant. The opposite can never occur. Therefore it is only relevant to calculate the EPV when a logistic regression has identified a significant odds ratio for a possible predictor. EPV was not calculated in Paper III since a positive urine culture in no case predicted symptoms.

The relationship between the proportion of positive tests in patients sick from another cause (the proportion of symptomatic carriers) and the proportion of positive tests in healthy individuals (the proportion of asymptomatic carriers) is defined as theta (θ) [126]. When evaluating infections it is reasonable to define that $\theta=1.0$; i.e. the found agent is linked to presented symptoms in a population as soon as the proportion of positive tests exceeds that of a healthy control population, thus θ was set to 1.0 in Paper II when calculating the EPV. However moderate alterations of theta does not cause any significant changes of the results [126, 136]. To calculate the EPV, the sensitivity of a urine culture to detect bacteriuria was estimated at 90%.

The effect of Simpsons paradox when evaluating dipsticks

It may seem peculiar that PPV for any bacteria was higher than PPV for a single bacterium (Tables 13-16) in Paper I. One explanation is that prevalence of bacteria in the gold standard is higher when the focus is on “any bacteria” compared to a specific bacterium, subsequently decreasing the probability of a false positive dipstick. The reverse was seen for NPV.

Another way to explain this phenomenon is the well-known Simpson’s paradox. Several potentially pathogenic bacteria differ in their ability to reduce nitrate to nitrite. Similarly, different bacteria are likely to show a varying ability to provoke pyuria. These differences are confounding factors, and as the prevalence of the different types of bacteria varies considerably, the size of the groups vary. This phenomenon has been previously explained as Yule-Simpson’s effect, a statistical paradox in which the outcome of several groups is changed when groups are combined [108-110].

5.4 Bacteriuria and nonspecific symptoms

In Paper III it is interesting to note that a positive urine culture was not commoner among residents with nonspecific symptoms compared to residents without symptoms. There was a trend ($p=0.057$) toward a lower proportion of positive urine cultures among residents with confusion occurring during the last month (Paper III, Table 1). This suggests that nonspecific symptoms are not caused by bacteria in the urine.

ABU increased with age and when an individual's general health declined [137]. When general health declined and elderly residents became frailer, they were more prone to cognitive decline, disability and symptoms such as fatigue [138, 139]. Accordingly, bacteriuria and nonspecific symptoms can simultaneously increase with no causality whatsoever. Hospitalized patients with dementia were more likely to have asymptomatic *E. coli* bacteriuria compared to patients without dementia [140]. Confusion was associated with dementing disorders [36]. Therefore, those with dementing disorders are more often confused and also more often bacteriuric without there necessarily being a causality between bacteriuria and confusion. A further example, urinary incontinence was more common among patients with ABU [5, 25]. In another study, urinary incontinence was associated with falls in institutionalized older adults [141]. Accordingly, as urinary incontinence is associated with higher incidence of ABU, those with falls are more bacteriuric without necessarily causality between bacteriuria and falls. This might explain why there were some statistical correlations at a group level between bacteriuria and some of the nonspecific symptoms, calculated by logistic regressions in Paper II.

When calculating predictive values to assess the clinical relevance of correlations at a group level in Paper II, only one EPV turned out to be possibly relevant. This was for a patient not being herself/himself present for at least one month, but less than three months, and growth of *E. coli* in the urine where the EPV was 96% (51-100%); that is the probability for *E. coli* to be associated to this symptom. This was *not* valid for "any bacteria", just for *E. coli*. Neither was it valid if only significant growth was defined as a positive urine culture. No other subset of duration of symptoms showed any clinically relevant EPV. It seems a bit odd that a symptom had to be present for at least one month to be an infection. This might be a type one error (a statistical significance by chance), as we did multiple comparisons in this study when evaluating different time intervals and different bacterial species. Furthermore, this single possibly relevant predictive value in Paper II had a wide confidence interval (51-100%). In conclusion, this finding is too unsure to be used in clinical practice; it has to be confirmed or rejected in an RCT. In all other situations,

findings of bacteria in the urine did not add any information and physicians must investigate the possibility of other explanations to the symptoms than lower UTI (Paper II). For the purpose of Paper III, we only analysed newly onset or increased symptoms during the last month and still present when the urine specimen was obtained. If, despite this, calculating the odds ratio for significant growth of *E. coli* to predict not being herself/himself for the preceding period of >1 month and <3 months from data gathered in 2012, there was no association (also valid for significant plus sparse growth of *E. coli*). This strengthens the hypothesis that the association found in Paper II between significant plus sparse growth of *E. coli*, and not being herself/himself for the preceding period of >1 month and <3 months was a type one error.

Bacteria in the urine could not predict any of the symptoms when calculating logistic regression in Paper III. On the contrary the adjusted odds ratio for bacteriuria to predict confusion was <1, i.e. positive urine cultures were less common among residents with confusion. A possible explanation for findings of statistical correlation at a group level between symptoms and bacteriuria in Paper II but not in Paper III might be that Paper III only analysed symptoms which had lasted up to one month. Those with symptoms lasting up to three months analysed in Paper II might, to a greater extent, represent a more frail population more prone to ABU as discussed previously.

Several previous studies suggest using the clinician's judgement as a gold standard to determine if bacteriuria is a symptomatic UTI or not, sometimes in combination with the outcome of dipstick urinalysis (discussed in 5.3.4 Lack of gold standard). However, using the clinician as the gold standard in studies trying to sort out a truth that is later to be used as a guideline for clinicians, is a circular argument. This thesis recognises that an appropriate gold standard for this situation is lacking and another approach not requiring a gold standard is needed. In this thesis we finally suggest that nonspecific symptoms such as confusion, fatigue, restlessness and aggressiveness are unlikely to be caused by bacteria in the urine (Papers II-III).

5.5 Urgency and dysuria

This thesis primarily aimed to study non UTI specific symptoms. As UTI specific symptoms were less frequent, these studies were partially underpowered regarding UTI specific symptoms. However, it is remarkable that there was no correlation between positive urine cultures and urinary frequency, urgency or dysuria in Papers II and III. This might be explained by the fact that there are many other causes of urgency and dysuria than UTI among residents of nursing homes [43-45, 142], and at the same time the high

prevalence of ABU in this population. Thus, urine cultures are not sufficiently specific in these situations.

In Paper III, it is interesting to note that among all symptoms urinary frequency was the only symptom where the proportion of positive urine cultures differed from those not having this symptom. Those with urinary frequency had a lower proportion of positive urine cultures and a trend (not significant) towards a higher proportion of having had antibiotic treatment during the previous month. Another explanation for this could be a shorter bladder incubation time in that group.

Newly onset dysuria, urinary urgency and frequency have validity as diagnostic criteria for symptomatic UTI as it is consistent with presentations of symptomatic UTI in other populations [39, 40]. However it is important to remember that chronic genitourinary symptoms are not due to UTI, although many residents with these symptoms are bacteriuric [2, 12].

5.6 Dipstick urinalysis and symptoms

There were no differences in urine dipstick analyses either for nitrite or leukocyte esterase $\geq 3+$ between residents with positive urine cultures when divided by the presence or absence of symptoms in Paper III. Subsequently urine dipsticks are not useful to differentiate between ABU and cystitis in residents with bacteriuria.

5.7 IL-6, bacteriuria and symptoms

Residents with positive urine cultures had higher concentrations of IL-6 in the urine (Paper III). However, among residents with positive urine cultures there were no differences in IL-6 concentrations between those with or without nonspecific symptoms. Thus IL-6 concentrations are not useful to differentiate between ABU and cystitis in residents with bacteriuria.

If nonspecific symptoms are not caused by bacteria in the urine, IL-6 concentrations cannot identify a subgroup of residents with more severe inflammation in the bladder correlating to nonspecific symptoms.

5.8 Further comparison with existing literature

5.8.1 Antimicrobial resistance

Several studies have shown increased antimicrobial resistance in uropathogens during the last ten years [77, 135]. In Paper IV, the average resistance rates for all tested antibiotics were similar between 2003 and 2012. In contrast to most countries the prevalence of ESBL-producing Enterobacteriaceae was low in residents in Paper IV. This may be partly due to successful efforts in Sweden to lower antibiotic usage and the choice of narrow spectrum antibiotics in favour of e.g. ciprofloxacin [70]. Thus, the annual number of antibiotic prescriptions per 1000 inhabitants over 65 years has decreased during the last years in Sweden [46].

The overall resistance rates in *E. coli* for UTI antibiotics in Sweden in 2012 [46], as compared to Paper IV (% R in Sweden / % R in Paper IV) were; for ampicillin 31% / 21%, mecillinam 4.6% / 7.7%, cefadroxil 3.5% / 2.6%, nitrofurantoin 1.1% / 0.85%, trimethoprim 19% / 12% and ciprofloxacin 7.6% / 3.4%. Thus, the proportion of resistant bacteria was lower than expected for all antibiotics excepting mecillinam in Paper IV. This might be partly explained by national statistics being based on clinical isolates, and our material from all those able to provide a urine specimen. On the other hand a nursing home population can be assumed to have higher antimicrobial resistance rates, since they have a high level of co-morbidity and are prescribed more antibiotics than younger individuals [84]. Thus, it is satisfying to note that a Swedish nursing home population still has comparatively low antimicrobial resistance rates among *E. coli*.

Hospitalization during the previous six months increased the risk for antibiotic resistance in *E. coli* against ampicillin, ciprofloxacin and any antimicrobial tested, adjusted for age, gender and any antibiotic treatments during the previous six months (Paper IV). This is consistent with several articles reporting prior hospitalization as a risk factor for antimicrobial resistance, which suggests that the hospital is a source of antibiotic-resistant organisms [84]. Those admitted to the hospital may also represent a group more prone to acquire antibiotic resistant bacteria due to the presence of decubitus ulcers, wounds, urinary incontinence, faecal incontinence and other hygienic risk factors.

5.8.2 Dipstick urinalysis (Paper I)

The study by Juthani-Mehta et al [99] presenting confidence intervals for PPV and NPV included only patients with symptoms of suspected UTI. Specific symptoms were dysuria (7%), changes in voiding patterns (6%) or fever (12%); nonspecific symptoms were changes in mental status (40%), behaviour (20%), changes in urine characteristics (17%), family or patient request (7%) and evaluation for other infections (7%) etc. However, it is unclear which clinical features or events are relevant to bacteriuria [1, 2, 116]. Thus, while Juthani-Mehta et al attempted to estimate the dipstick analysis' reliability in predicting UTI, Paper I focused on evaluating the dipstick's reliability to predict bacteriuria, not UTI. Prevalence of bacteriuria among asymptomatic residents of nursing homes for the elderly is high [2, 5, 25] and similar to the prevalence found by Juthani-Mehta et al and as reported in Paper I. Since PPV and NPV for dipstick urinalysis depend on prevalence of bacteriuria there should be no major differences between evaluating dipstick analyses for symptomatic or asymptomatic individuals.

The conclusion that when dipstick urinalyses for nitrite and leukocyte esterase are simultaneously negative it is unlikely that a urine culture will show growth of potentially pathogenic bacteria was based on "any bacteria" in Paper I (Table 16). Furthermore, the NPV for each single bacterium was higher, thus making the conclusion appear valid. A previously published meta-analysis assumed that a positive nitrite test could rule in bacteriuria in the elderly [100]. However, we found that PPV for "any bacteria" differs considerably from PPV for the single bacteria in Paper I (Table 14). Thus, the conclusion made in the meta-analysis that a positive nitrite dipstick can rule in bacteriuria seems unjustified.

5.8.3 Antibiotic treatment and negative urine culture

In Paper III, residents with recently onset confusion, loss of appetite, frequent falls and any of the nonspecific symptoms had oftener been prescribed antibiotics during the last month. This might explain the trend toward the lower prevalence of bacteriuria among residents with confusion. Also, in the logistic regressions, antibiotics during the previous month were a predictor of loss of appetite, frequent falls and "any of the nonspecific symptoms". This supports previous studies showing that nonspecific symptoms were a common reason for suspecting UTI and the prescription of antibiotics [32, 34, 35, 37]. These registered symptoms in Paper III might also reflect side effects of prescribed antibiotics as the elderly are more likely to retain side effects from antibiotics [75]. These residents could also represent a frailer population having more

nonspecific symptoms, and also being more prone to infection, and consequently more antibiotic prescriptions.

Even if this thesis strongly suggests that nonspecific symptoms are not caused by bacteria in the urine, due to the possible confounders described above, the best proof would be a future randomized controlled trial evaluating UTI antibiotic treatment of nonspecific symptoms among elderly residents of nursing homes.

5.9 Implications for clinical practice

Changes in mental status such as confusion and fatigue are the most common reason for suspecting UTI in nursing homes. However, this thesis shows that nonspecific symptoms such as confusion, fatigue, restlessness and aggressiveness are highly unlikely to be caused by bacteria in the urine.

It would have been most convenient just to prescribe a UTI antibiotic to an elderly resident with bacteriuria and a newly onset nonspecific symptom. However, not initially considering other more plausible causes of the symptom places the patient at risk for having other undiagnosed conditions. The causes could be too much medication, drug dosages too high for an elderly resident, interaction between drugs, having a “bad day”, having had a visit the day before, new staff at the nursing home, dehydration, obstipation, other diseases, and so forth. If there is a small percentage that nevertheless has a symptomatic UTI dipstick urinalysis, urine IL-6 and urine cultures are not specific enough and a proper diagnostic method remains undetermined.

Nonspecific symptoms, in the absence of urinary tract symptoms, all too often lead to antibiotic treatment with the excuse that it is impossible to know if an elderly resident has urinary tract symptoms or not. There has obviously been a urine specimen sampled if the outcome of dipstick urinalysis or a urine culture is known. If capable of providing a urine sample without assistance, the resident could probably also tell if urinating was painful or unpleasant. If the resident suffered from dementia too severe to provide the urine specimen alone, a member of the staff assisted. In these situations the personnel can observe the resident if urination seems painful. It is also possible to observe if there is frequent urination. Thus, it is possible to go further in the diagnostic process. However, nonspecific symptoms in the absence of urinary tract symptoms are unlikely to be a lower UTI.

Although there are causes other than lower UTI for symptoms from the urinary tract, these symptoms are more likely to be a lower UTI if they are of recent

onset. Compared to nonspecific symptoms, symptoms from the urinary tract are a minor reason for suspecting UTI in residents of nursing homes for the elderly. Therefore, antibiotic prescriptions in nursing homes would decrease significantly if prescriptions were to be reduced for nonspecific symptoms, but without changing prescription patterns for residents with new urinary tract symptoms. However, it is important to remember that long-lasting unchanged symptoms from the urinary tract are not usually a lower UTI. A rational use of antibiotics and improving antimicrobial stewardship is an international priority due to the evolving threat of antibiotic resistance.

6 CONCLUSIONS

The results of this thesis can be considered valid in ordinary clinical practice when evaluating elderly residents of nursing homes with new or increased nonspecific symptoms and possible bacteriuria.

Recently onset nonspecific symptoms were common among elderly residents of nursing homes. Residents without nonspecific symptoms had positive urine cultures as often as those with nonspecific symptoms with a duration of up to one month, suggesting that nonspecific symptoms are unlikely to be caused by bacteria in the urine. We therefore conclude in this thesis that urine cultures provide little or no useful information when evaluating nonspecific symptoms among elderly residents of nursing homes.

Residents with positive urine cultures had higher concentrations of IL-6 in the urine. However, among residents with positive urine cultures no differences in IL-6 concentrations between those with or without nonspecific symptoms were found. Thus, IL-6 concentrations are not useful when assessing elderly residents with nonspecific symptoms and bacteria in the urine. Neither are urine nitrite and leukocyte esterase dipsticks useful in differentiating between ABU and UTI in residents with bacteriuria.

If there is a reason for testing for bacteriuria, this thesis may provide some simple guidelines. When dipstick urinalyses for nitrite and leukocyte esterase are simultaneously negative it is unlikely that a urine culture will show growth of potentially pathogenic bacteria. Thus, in a patient with an uncomplicated illness, no further testing is needed. However, a positive dipstick or any combination thereof cannot reliably rule in bacteriuria.

When dipstick urinalysis was performed in nursing homes for the elderly by non-laboratory personnel, there were no clinically relevant differences between visual and analyser readings of dipsticks. Thus, the choice between visual and analyser reading could be based on personal preference and economic aspects.

There were still comparatively low levels of antimicrobial resistance in urinary pathogens among Swedish nursing home residents with no major changes between 2003 and 2012. Any antibiotic treatment during the last month and hospitalization during the last six months predicted higher resistance rates in *E. coli*. Due to the potentially high risk for increasing antibiotic resistance in this population, it is important to use antibiotics rationally to preserve the efficacy of treating patients with existing antibiotics.

7 FUTURE PERSPECTIVES

Although this thesis suggests that nonspecific symptoms are not caused by bacteria in the urine, there are possible confounders and limitations described in the discussion of this thesis. A possible way to resolve this would be a large randomized controlled trial to test if there are any differences in symptom follow-ups between residents receiving placebos and UTI antibiotics, respectively. However, an RCT in a large population of fragile elderly individuals, many with dementia and no possibility to give statement of consent would be very difficult. Due to the inherent difficulties of such a study it would be advantageous to carry out a broad multicentre study, possibly with international collaboration.

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