University of Gothenburg

Urinary tract infections

– Etiology, antibiotic susceptibility, and treatment in surgical patients in Nepal

UNIVERSITY OF GOTHENBURG

Master thesis in Medicine

Programme in Medicine

Gothenburg, Sweden 2014

Adam Oscarson

Supervisors:
Dr Leif Dotevall
Dr Pukar Maskey
Dr Amit Arjyal
Dr Poojan Shrestha

Table of Contents

ABSTRACT

Master thesis, Programme in Medicine.

Urinary tract infections – Etiology, antibiotic susceptibility and treatment in surgical patients in Nepal. Adam Oscarson, University of Gothenburg, Sweden 2014.

Background: Urinary tract infections (UTI) are among the most common postoperative nosocomial infections and the second most common reason for empirical antibiotic treatment. Optimal treatment could decrease mortality and morbidity in surgical patients and play a vital role in combating the ongoing crises of increasing antibiotic resistance.

Aims: To study the pathogens and their antibiotic susceptibility in urinary cultures from a surgical clinic in a tertiary care hospital in Nepal; and to study the use of antibiotics, rationality of antibiotic treatment and surgical risk factors for urinary tract infection.

Methods: All urinary cultures from the surgical clinic during 18 month were reviewed. In addition, 50 patients had their medical chart reviewed for treatment and risk factors.

Result: *E. coli* was the most common bacteria (74 %). The overall level of resistance to commonly used antibiotics were high in all pathogens. Amikacin and nitrofurantoin were generally the antibiotics with lowest rates of resistance. Aminoglycosides and fluoroquinolones were the most often used antibiotics. In first-line treatment, only 55 % of cases were given at least one antibiotic to which the bacteria was sensitive. A statistical significant higher resistant to both ciprofloxacin and cefotaxime were found in cultures from the surgical ward compared to the surgical reference clinic ($P = 0.02$ and $P = 0.002$), and to ciprofloxacin in cases with indwelling urinary catheter compared to cases without indwelling

urinary catheter (P = 0.005) and in cases with previous admittance to hospital compared to cases without previous admittance to hospital (P = 0.008).

Conclusions: This study found an exceptional high rate of antibiotic resistance, and a low rate of effective empirical antibiotic treatment. Guidelines on antibiotic treatment are needed, and for empirical treatment of nosocomial UTI an increased use of nitrofurantoin and amikacin is recommended, rather than cephalosporins and fluoroquinolones.

BACKGROUND

Antibiotic resistance

Antibiotic resistance (ABR) is a normal evolutionary process. In environments where antibiotics are abundant and thus constitute an important selection pressure, mutations that entail resistance provides a competitive advantage to the microbes carrying it and are therefore favoured in the natural selection. The vast use of antibiotics in hospitals and the community – as well as in agriculture – over the last decades have accelerated this process to a point where ABR have emerged as a global health problem. Widespread resistance to antibiotics is not only creating difficult-to-treat infections associated with high mortality, but also threatening major progresses in modern medicine like major surgery and cancer chemotherapy, as well as having social and economic impact [1, 2]. Bacteria with resistance to broad-spectrum antibiotics are increasing, and with the emergence of multidrug-resistant bacteria there is an increasing fear that we are heading towards a post-antibiotic era [2-4]. Resistance to antibiotics are now found all over the world, and have even been found in the Arctic – a region without selection pressure for resistance development [5]. Resistance is spread between bacteria, humans and regions by means of transmission of resistance genes between bacteria (e.g. plasmids and transposons), poor sanitation and hygiene in hospitals and communities, and global travel, trade and migration.

Since the turn of the millennium, bacteria of the family *Enterobacteriaceae* producing extended-spectrum β-lactamases (ESBL) – enzymes providing resistance to almost all β-lactam antibiotics (like penicillin and cephalosporins) except carbapenems – have emerged as an important cause of infections, including urinary tract infections (UTI). They have since spread to all over the world and are often associated with multi-drug resistance including resistance to co-trimoxazole, aminoglycosides and ciprofloxacin – important antibiotics in the

(empirical) treatment of UTI [6]. Since a few years back, we also see an alarmingly rapid world-wide dissemination of resistance against carbapenems [7]. One example is the New Delhi metallo-β-lactamase 1 (NDM-1) gene, first identified in Sweden 2008 in a urine culture from a patient previously hospitalized in New Delhi [8]. Within a few years, the gene had spread to large parts of the world [9-11]. The NDM-1 gene is exhibiting several worrisome characteristics like being associated with plasmids carrying high number of genes mediating resistance to virtually all antibiotics but tigecycline, colistin and fosfomycin, as well as being found in many unrelated species of bacteria including *Klebsiella pneumoniae* (a common nosocomial pathogen) and *Escherichia coli* (the main community-acquired human pathogen) [9, 12], also the two main pathogens of UTI [13].

Selection of resistance genes and spread of bacteria with ABR is mainly a local process, with practices in individual hospitals and communities playing a critical role [2]. Resistance genes may be transferred to bacterial spices capable of causing other kinds of infections than the original one. Successful genes may then be further selected and transferred to new hosts, and in that process being amplified and established as important resistance genes, especially if the selection pressure of antibiotics continues. They can then spread through different kinds of bacteria nearly everywhere through the world's interconnecting commensal, environmental, and pathogenic bacterial populations. As an example, resistance to sulfonamides has been found throughout the world encoded by only 2 resistance genes [14].

The combination of highly susceptible patients, intensive and prolonged antibiotic use and cross-infections make hospitals a hotspot for both evolution and spread of ABR. Emergence of resistant strains in hospitals occurs when a patient infected with a resistant bacteria is transferred to the hospital from another facility, by patient-to-patient transfer, through

selection caused by antibiotic use, and by transfer of resistance genes. Overcrowding, limited capacity and poor sanitation make the situation worse in many low and middle-income countries (LMICs). Healthcare-associated infections are a problem worldwide, adding not only to the burden of both infections and ABR but also constituting a major threat to patients' safety. A review [15] found the pooled prevalence of overall healthcare-associated infection in LMICs to be 15.5 per 100 patients, twice as much as in Europe (7.1 per 100 patients) [16]. Gram-negative bacteria represented the most common isolates in nosocomial infections.

The rate of resistance varies widely between bacteria, class of antibiotic, countries, patient categories and healthcare facilities. Different levels of resistance between and within countries might be explained by differences in consumption [17]. Worldwide the antibiotic consumption is increasing, including carbapenems [2]. The use of non-prescription antibiotics are common in many parts of the world and outside of northern Europe and North America non-prescription use account for 19 - 100 % of antimicrobial use [18]. Increasing non-prescription use of antibiotics has been associated with clinically important ABR, including high levels of resistance in *E. coli* in urinary cultures [18-20]. In countries with limited and/or poor access to healthcare, a ban on over-the-counter antibiotics might impede vital access to antibiotics. Especially in LMICs this provides a dilemma on how to ensure access to antibiotics without excessive or inappropriate use. The access and excess dilemma is however not restricted to non-prescription use (or to the LMICs) since prescribed antibiotic could be unnecessary or suboptimal, or patients in need are not given treatment due to therapeutic, financial and structural barriers. As an example, less than a third of children in LMICs with suspected pneumonia receive potentially life-saving treatment with antibiotics at the same time as antibiotics are too often prescribed for diarrhoea instead oral rehydration salts and zinc [21, 22].

Both the prevalence of ABR around the world, and the burden of it, is inadequately studied [1, 2]. Most studies are carried out in a hospital setting, often with cultures from severely ill patients where first-line treatment have failed. Community-acquired infections and infections treated in out-patient-care are underrepresented. This may exaggerate the rates of ABR and further drive the use of broad-spectrum antibiotics, which in turn may further accelerate the evolution and spread of resistance [1]. The burden of ABR is thought to the result of longer duration of illness and higher mortality, increasing costs of treatment and inability to perform procedures that require antibiotics to prevent infections [2]. An often cited study from 2007 [23] estimated that each year about 25 000 patients in the European Union, Iceland and Norway die from an infection with resistant bacteria, and that ABR result in extra healthcare costs and productivity losses of at least EUR 1.5 billion each year. Although poorly studied, the higher prevalence of infectious diseases and restricted access to healthcare and second-line antibiotics in LMICs, it is estimated that the burden probably is higher than in high-income countries, and since two thirds of childhood mortality is associated with infections, children are probably more affected than adults [2, 24]. Adding onto the already complex issue of ABR, the situation in many LMICs is often further aggravated by poverty, which leads to poor sanitation, hunger and malnutrition, inadequate access to drugs, poor and inadequate health care systems, civil conflicts and bad governance [25]. Also, the problem of resistance might not be adequately recognised by most stakeholders as there are higher priorities to address such as provision of basic health care or sanitation.

As have been pointed out above, we are now in a situation where ABR is found worldwide, and in many places constitute an important medical problem. There is no single, easy solution to this problem, but rather a need for a multitude of actions and strategies. Effective infection-control practices and good hygiene are the foundation in controlling the spread of

antimicrobial resistance in healthcare settings. In communities, reduction in infections and decreased colonisation and transfer of resistant bacteria and resistance genes can be achieved through reduction of poverty, improved sanitation and access to clean water [2]. Improvement in rational use of antibiotics through so called antibiotic stewardship programs with education, treatment guidelines, restricted availability to certain antibiotics, surveillance of ABR and antibiotic usage, and post prescription audit and feedback of antibiotic use, can decrease the emergence of antimicrobial resistance and reduce colonization or infection with resistant bacteria [26, 27]. As an example, a study [28] done in a university-affiliated hospital found that a new antibiotic guideline that reduced the use of cephalosporins by 80 % led to a 44 % hospital-wide decrease in ESBL-producing *Klebsiella* isolates within 1 year. Antibiotic stewardship programs should also be expanded to LMICs, primary care settings and the community, and adjusted to local conditions. Where prescriptions of antibiotics are a source of revenue – either for individuals or institutions such as hospitals – efforts should be made to separate prescription and dispensing [2]. New antibiotics needs to be developed. Since the 1970:s only two new classes of antibiotics have reached the market, and the number of new analogues are decreasing. At the moment though, the pipeline for new antibiotics is almost dry [2]. However, resistance will eventually arise to all antibiotics, so new antibiotics needs to be used with caution and be paired with effective stewardship programmes. To avoid pharmaceutical companies maximising profits on new antibiotics by selling large quantities, delinkage of revenues and the use of the product is needed and instead new models of income needs to be developed, as well as models permitting global access at affordable prices [2, 29] . In the meantime – while we wait for new antibiotics to appear – new approaches are needed to address the problem. Studied today are: anti-virulence approaches, phage therapy, therapeutic antibodies, drugs based on antimicrobial peptides, potentiators of traditional antibiotics (like

the β-lactamase inhibitors already in clinical use) and efflux pump inhibitors, and antibacterial biomaterials [30].

Antibiotic resistance and its consequences a serious threat to public health akin to that of anthropogenic global warming, and calls for concerted global action. We need a worldwide coalition of governments with a strong representation from LMICs, WHO and other UN agencies, other international bodies, science academies, development aid agencies, civil society organisations and pharmaceutical companies to develop a global plan to tackle the antibiotic crisis.

Urinary tract infections

Urinary tract infections are among the most common bacterial infections in humans, both as community-acquired and healthcare-associated infections. It is the most common nonsurgical nosocomial infection in postoperative patients and the second most common healthcare-associated infection [15, 31]. As the second most common reason for empirical antibiotic treatment, UTI is a major driver of antibiotic usage globally [32]. It have a wide spectrum of severity – and even though a usually benign infection – the occurrence of a UTI in a surgical patient is associated with a threefold increase in death during hospitalization [33]. Hence, the prevention and treatment of UTI is therefore of great concern for the survival and wellbeing of the individual surgical patient.

Bacteriuria – the presence of bacteria in the urine – can be subdivided into the following categories and definitions [13, 32]:

- *Asymptomatic bacteriuria* (ABU) – bacteriuria in the absence of symptoms. Should generally not be treated.

- *Symptomatic bacteriuria* – bacteriuria in the presence of typical symptoms for UTI.
- *Lower UTI* – the infection is localized in the lower urinary tracts, i.e. the bladder and urethra. Fever is uncommon and the general condition of the patient is usually unaffected.
- *Upper UTI* – the infection is localized to the upper urinary tracts, also called pyelonephritis; a much more severe condition than lower UTI, and frequently causes sepsis (urosepsis).
- *Complicated UTI* – upper UTI and/or UTI in individuals with predisposing factors such as structural and functional abnormalities in the urinary tracts, metabolic disorders or impaired immunity. UTI in children and men are often considered to be complicated UTI, and are more often caused by multi-resistant organisms.

E. coli is by far the most common pathogen, often accounting for more than 80 % in uncomplicated UTI, although most studies are done in high- or middle-income countries. Other pathogens (so-called secondary pathogens such as *Klebsiella spp, Enterobacter, Proteus spp*) and are usually found in patients with complicated UTI. Generally, these pathogens have a decreased susceptibility to many antibiotics [13].

Predisposing factors for UTI include structural and functional abnormalities, foreign bodies, metabolic abnormalities, impaired immunity and urological surgery and instrumentation [32]. Indwelling catheter use is a known risk factor for UTI, with duration of catheterization as the single most important risk factor [32].The bacteria is often of several different species, often multi-resistant [13]. Catheter-associated UTI make up a large proportion (approximately 30 - 40 %) of the healthcare-associated infections, and as many as 10 % of patients in a urological wards have healthcare-associated complicated infections [32].

Nepal

Nepal is a small country in Asia, situated on the southern slopes of Himalaya. It is one of the world's poorest countries, ranking 157th out of 187 countries on the Human Development Index. A third of the population lives below the poverty line, and the income gaps are huge. It is also one of the world's least urbanized countries. Instability and political unrest have afflicted Nepal for decades, and a decade-long civil war ended in 2006. The public health situation is deficient with widespread infectious diseases, high infant mortality rate (46/1 000 in 2013) and a shortage of healthcare resources and staff. However, despite the numerous challenges, significant improvements in both poverty reduction and health have been made in the last decades [34, 35].

Studies and reports done in hospital settings (with a presumed dominance of complicated UTI) in Nepal shows a high degree of resistance in uropathogens: 19.2 % - 48 % for *E. coli* and *Klebsiella spp* resistant to third generation cephalosporins [36-38], 21.6 % - 35 % for *E. coli* and *Klebsiella spp* resistant to fluoroquinolones [36, 38]. These findings are in line with figures from WHO, however they reported a resistance rate of *E. coli* to fluoroquinolones of 64.3 % [1].

AIMS

This is a hospital-based retrospective descriptive study in two parts that aims to study:

1) Pathogens and antibiotic susceptibility among patients with presumed complicated UTI at a surgical clinic in a tertiary care hospital in Nepal.
2) The use of antibiotics, rationality of antibiotic treatment and surgical risk factors.

METHODS

The study was performed at Patan Hospital, a tertiary care hospital located in Patan (adjacent to Kathmandu), Nepal. It is one of the largest hospitals in the country, treating 320 000 outpatients, 20 000 inpatients and performing 10 000 operations annually. It is run by Patan Academy of Health Sciences, an autonomous, not-for-profit public institution of higher education. It accepts patients from all over the country offering subsidized or free treatment to patients without the ability to pay full price for the healthcare given, thus focusing on Nepal's rural poor [39].

In Part 1, the log books of urine cultures at the microbiological department were searched manually and all records of urine cultures from either the surgical ward or the surgical reference clinic from 2069-03-15 to 2070-09-15 according to Nepali calendar (2012-07-01 to 2013-12-31 in Gregorian calendar) were noted. A total number of 774 cultures from 715 unique patients were found. Since no record of whether the urine sample consisted of mid-stream or catheter urine were kept, it was not possible in the first part of the study to differentiate between these two procedures of urine culture. Growth with more than 10 000 colony forming units/ml (CFU/ml) were recorded by the laboratory and thus included in the study. Coagulase negative staphylococci, non-haemolytic streptococci and yeast cells were regarded as contamination. No test for ESBL was performed. Comparisons with results from blood cultures were not done.

For Part 2, all cultures with a growth of more than 100 000 CFU/ml and from a patient with a six digit hospital number (which means that the medical chart were kept in the hospital's archive) during the study period where then selected for review of their medical chart. With this criteria 70 cases were found (a case was defined as an independent illness episode), and

of these 50 cases (71 %) of 48 unique patients (two patients occurring two times) were found and retrieved in the hospital's archive; representing 37 % of cultures with significant growth and 6 % of total cultures. In accordance with other studies from Nepal [36-38], and local hospital definition of UTI, over 100 000 CFU/ml were considered significant growth and hence this definition is used in this part of the study.

The surgical ward at the hospital provided care for in-patients requiring 24-hour medical attention, while the surgical reference clinic treated out-patients coming to appointments with the doctor, e.g. postoperative follow-ups. From the surgical ward came 39 % (301 cultures) of total cultures, 32 % (44 cultures) in Part 1 and 40 % (20 cases) in Part 2, and from the surgical reference clinic came 61 % (473 cultures) of total cultures, 68 % (92 cultures) in Part 1 and 60 % (30 cases) in Part 2.

The distribution of *E. coli* were 74 % (101 cultures) in Part 1 and 72 % (36 cases) in Part 2; for non-*E. coli* cultures those numbers were 26 % (35 cultures) and 28 % (14 cases), respectively. The relatively good correlation between total cultures, Part 1 and Part 2 could indicate that the cases in Part 2 compose a representative sample. The large dropout rate was however a major limitation.

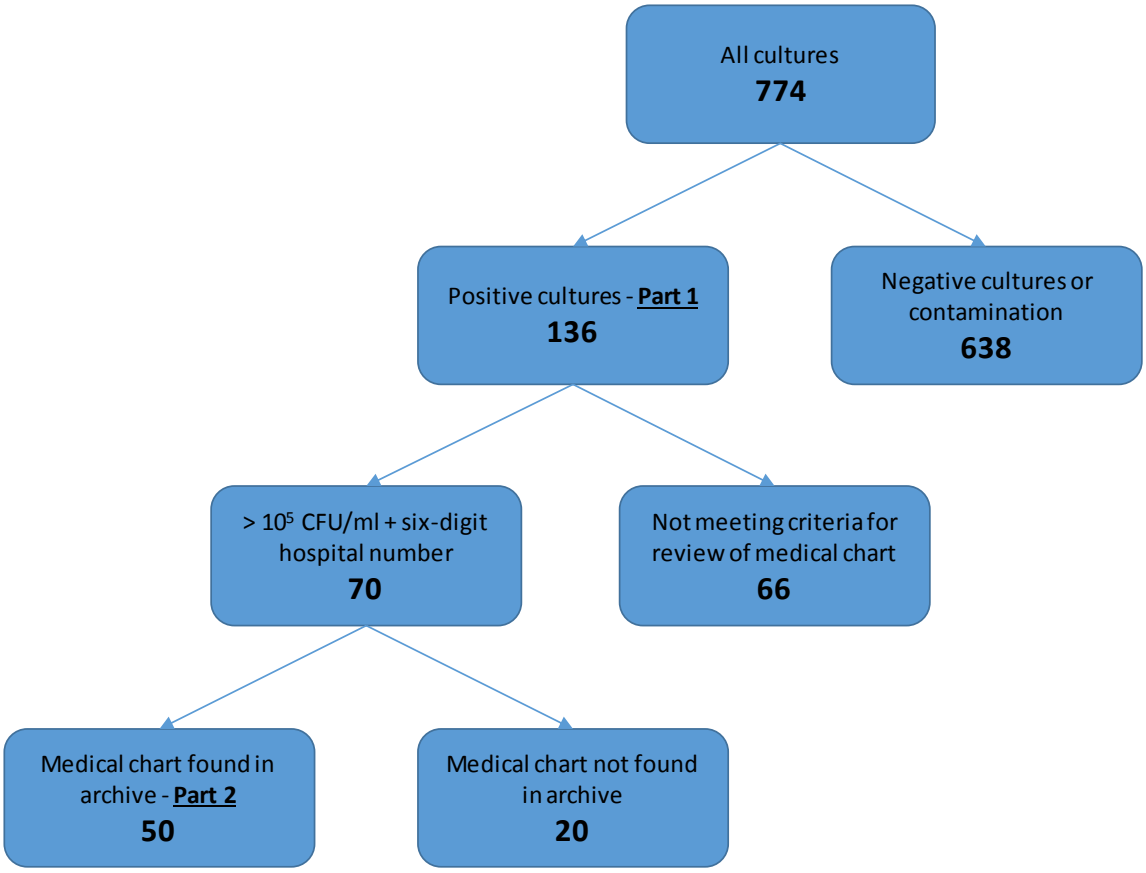

Figure 1. Data collection procedures. See text for more information.

Patients in Part 2

The number of female cases was 22, and male cases 28. Distribution of age and sex is presented in figure 2 below. No interviews were done with the patients, and all patient data are derived from medical charts. From the surgical ward came 20 cases (40 %) and from the surgical reference clinic 30 cases (60 %).

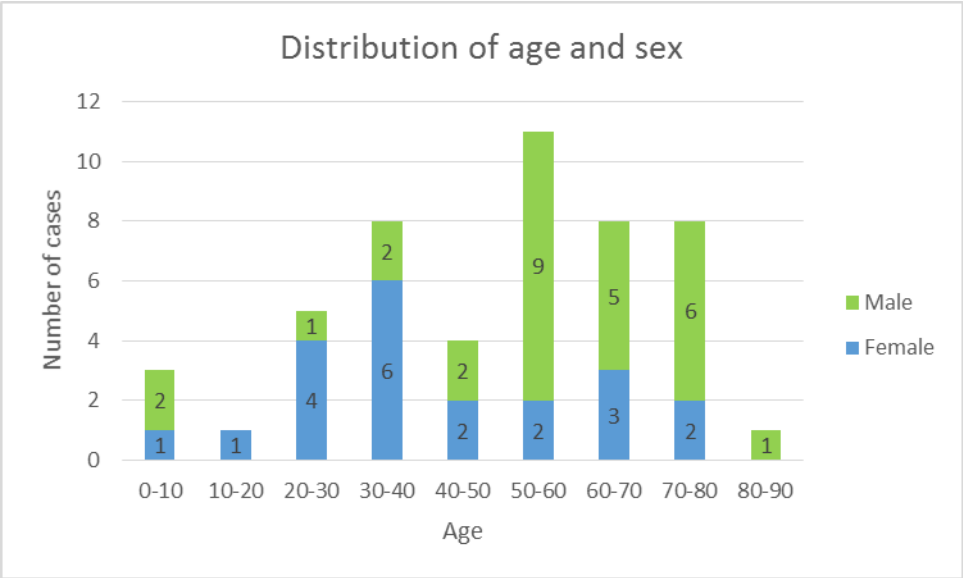

Figure 2. Distribution of age and sex. Numbers are number of cases. One female patient was lacking information about age and is excluded in this figure.

The urine samples were inoculated onto agar plates (blood agar and MacConkey agar) using calibrated loop. After incubation the plates were examined for bacterial growth and quantified. For positive cultures, bacterial identification was done using morphological characteristics, Gram staining and various biochemical tests. The sensitivity testing was done using the Kirby-Bauer disk diffusion susceptibility test.

Statistics

All documentation in the hospital were handwritten and in English. Information from the logbook of the laboratory was transferred into Microsoft Access 2013, and information from the medical charts was noted in Microsoft Excel 2013. Analyses were done in Microsoft Excel 2013 and all numbers have been rounded off to integers. Statistical analyses for P-value, risk ratio and 95 % confidence limits were done by chi square test with the software OpenEpi: Open Source Epidemiologic Statistics for Public Health [40].

ETHICS

Ethical approval was given by the Institutional Review Committee of Patan Academy of Health Sciences and the form is attached as Appendix A.

RESULTS

Part 1

Antibiotic susceptibility

In Part 1, 136 cultures (18 %) with nine different species of bacteria were found (figure 3). The most common pathogen was *Escherichia coli* (*E. coli*, 74 %), the second most common was *Klebsiella spp* (12 %). Of all cultures, 110 (14 % of total) had more than 100 000 CFU/ml, 75 % of those were *E. coli* (69 % from the surgical ward, 77 % from the surgical reference clinic [no statistical significance]). Worth noting is that no culture of *Salmonella spp* were found, despite being a common cause of blood stream infections in Nepal [41, 42].

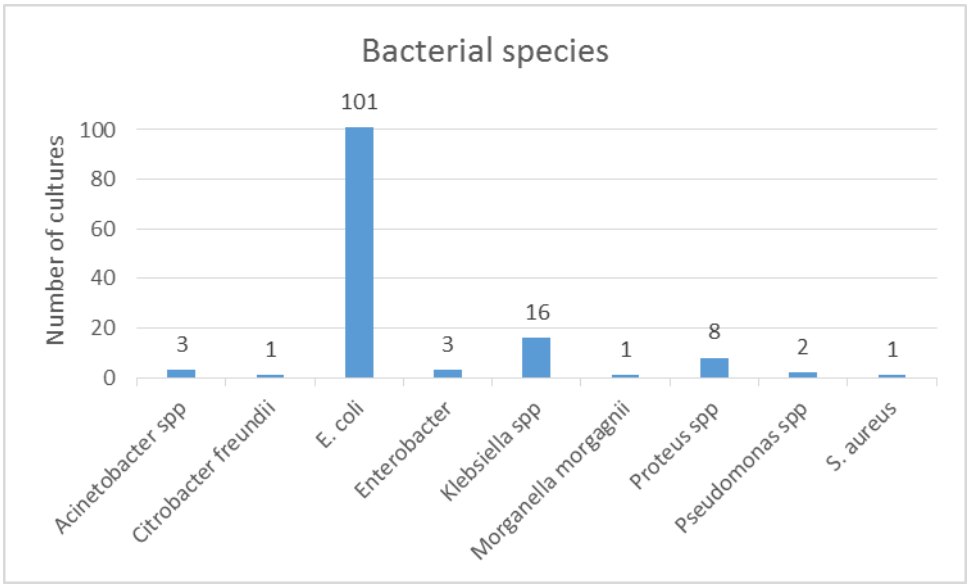

Figure 3. Bacterial species in positive cultures. Numbers are number of cultures, in total 136 cultures. S. aureus - Staphylococcus aureus.

The mean susceptibility of all the cultures combined is presented in figure 4. One culture each of *E. coli* and *Acinetobacter spp* have been omitted since susceptibility tests were not performed on these, giving a total of 134 cultures.

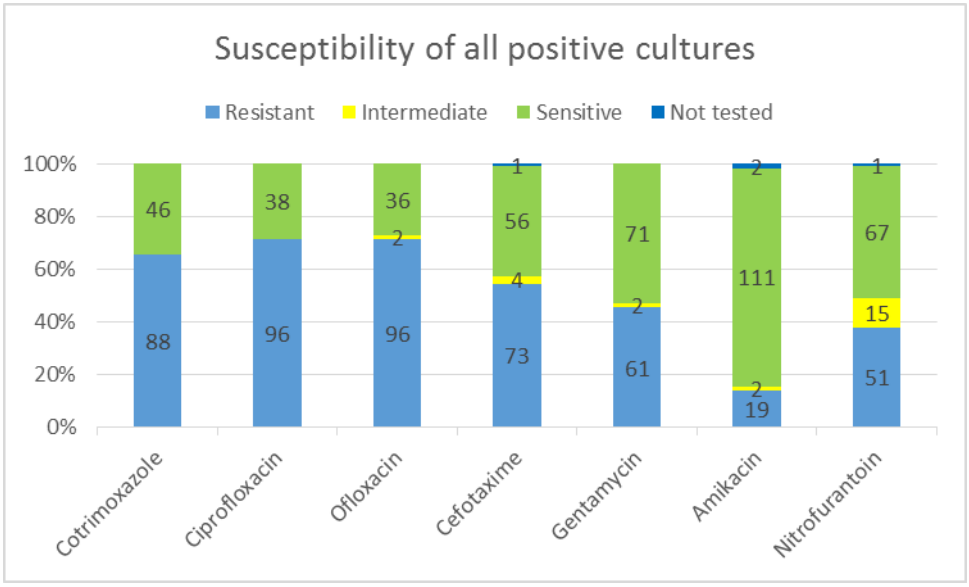

Figure 4. The susceptibility of all cultures combined to common antibiotics. Numbers are number of cultures. Not tested - no susceptibility test done.

Cultures with a high degree of resistance to common antibiotics were tested for further antibiotics (table 1). It is however important to notice that these antibiotics were only tested on a small number of cultures.

Susceptibility of all positive cultures				
	Resistant	**Intermediate**	**Sensitive**	**Total**
Meropenem	4 (40 %)	2 (20 %)	4 (40 %)	10
Imipenem	4 (36 %)	1 (9 %)	6 (55 %)	11
Piperacillin-Tazobactam	3 (75 %)	0	1 (25 %)	4
Colistin	1 (11 %)	0	8 (89 %)	9
Tigecycline	1 (17 %)	0	5 (83 %)	6

Table 1. Extended susceptibility testing of all cultures combined. Numbers are number of cultures.

The susceptibility of *E. coli* to common antibiotics is shown in figure 5. For one culture of *E. coli* no susceptibility test was done, and this culture have been omitted from further analyses. Hence, the susceptibility analyses are based on 100 cultures of *E. coli*. Table 2 shows the extended susceptibility testing.

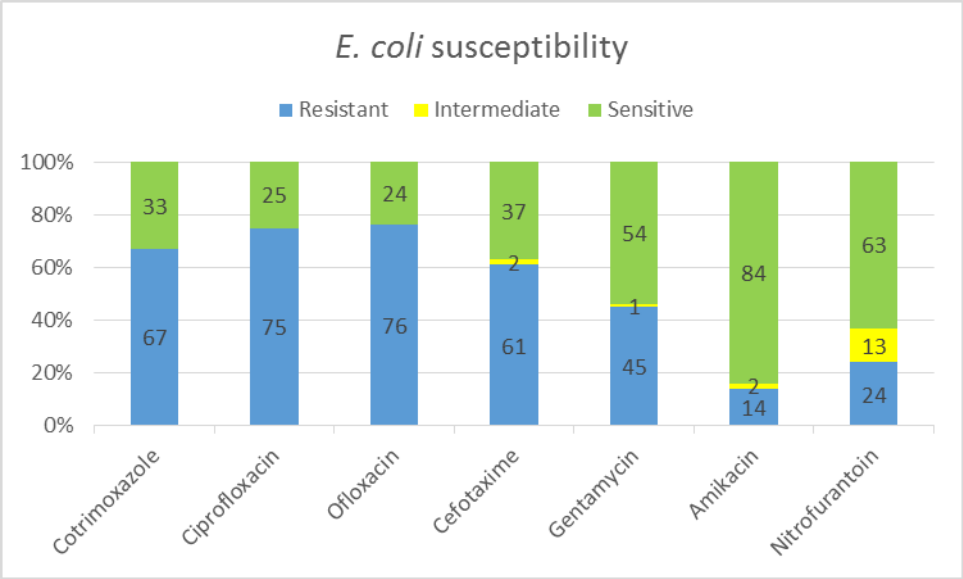

Figure 5. The susceptibility of E. coli to common antibiotics. The digits in the graph show the number of cultures.

***E. coli* susceptibility**				
	Resistant	**Intermediate**	**Sensitive**	**Total**
Meropenem	3 (50 %)	0	3 (50 %)	6
Imipenem	4 (57 %)	0	3 (43 %)	7
Piperacillin-Tazobactam	2 (67 %)	0	1 (33 %)	3
Colistin	1 (17 %)	0	5 (83 %)	6
Tigecycline	0 (0 %)	0	3 (100 %)	3

Table 2. Extended susceptibility testing of E. coli.

Figure 6 shows the susceptibility to common antibiotics of *Klebsiella spp*, and table 3 shows the extended susceptibility testing.

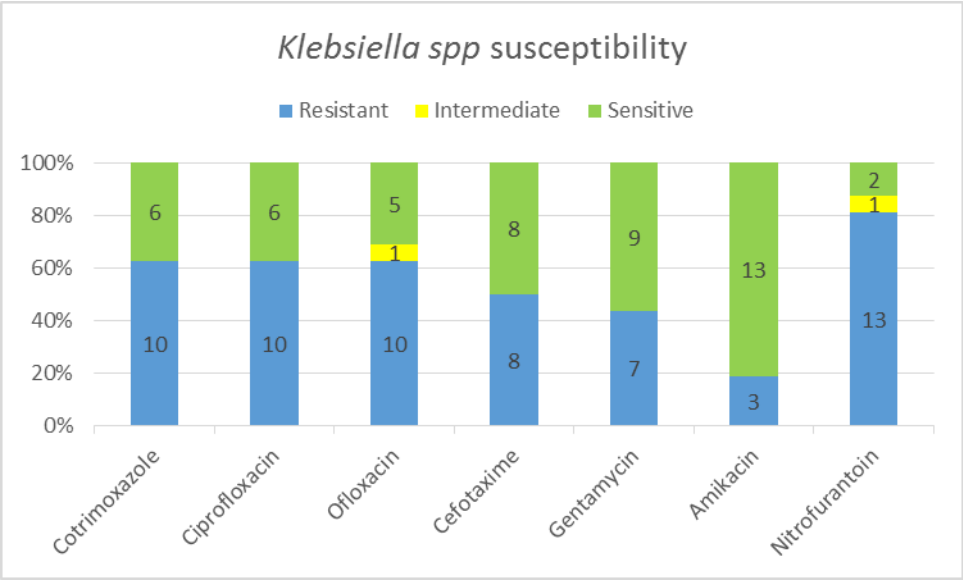

Figure 6. The susceptibility of Klebsiella spp to common antibiotics. The digits in the graph show the number of cultures.

***Klebsiella spp* susceptibility**				
	Resistant	**Intermediate**	**Sensitive**	**Total**
Meropenem	0	2 (67 %)	1 (33 %)	3
Imipenem	0	1 (33 %)	2 (67 %)	3
Piperacillin-Tazobactam	1 (100 %)	0	0	1
Colistin	0	0	2 (100 %)	2
Tigecycline	0	0	2 (100 %)	2

Table 3. Extended susceptibility testing of Klebsiella spp.

Susceptibility of non-*E. coli* pathogens combined are presented in figure 7 and table 4. One culture of *Acinetobacter spp* have been omitted since susceptibility test were not performed, giving a total of 34 cultures.

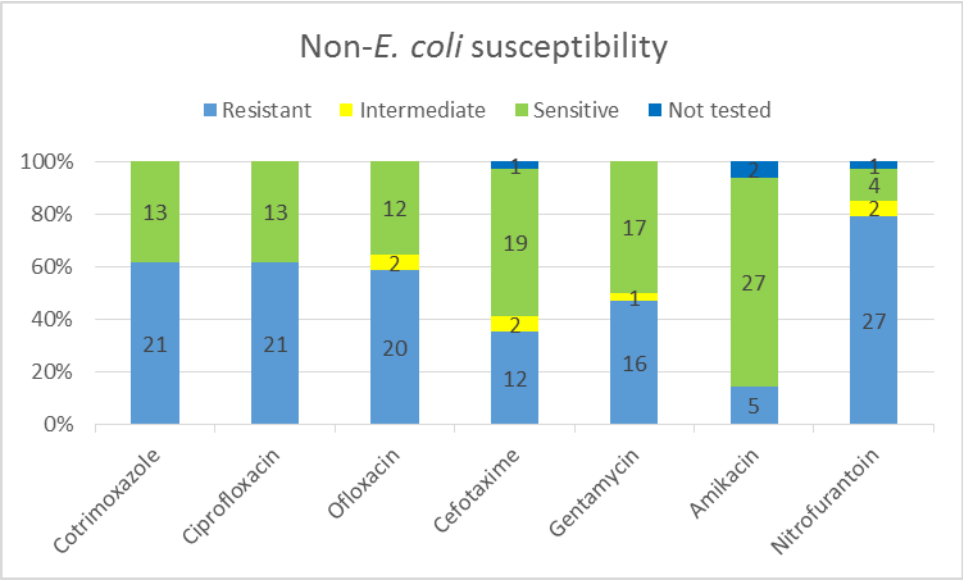

Figure 7. The susceptibility of non-E. coli pathogens to common antibiotics. The digits in the graph show the number of cultures. Not tested - no susceptibility test done.

Non-*E. coli* susceptibility				
	Resistant	**Intermediate**	**Sensitive**	**Total**
Meropenem	1 (25 %)	2 (50 %)	1 (25 %)	4
Imipenem	0	1 (25 %)	3 (75 %)	4
Piperacillin-Tazobactam	1 (100 %)	0	0	1
Colistin	0	0	3 (100 %)	3
Tigecycline	1 (33 %)	0	2 (67 %)	3

Table 4. Extended susceptibility testing of non-E. coli pathogens.

Further analyses were done to investigate the susceptibility of all the tested cultures to the commonly used antibiotics in treatment of UTI at PH [43]. Cultures not tested for some or any of the drugs have been omitted. The empirical treatment of uncomplicated UTI is usually fluoroquinolones such as ciprofloxacin and ofloxacin, or sometimes cefixime (a third generation cephalosporin). For complicated UTI, empirical treatment consists of either fluoroquinolones, third generation cephalosporins (usually ceftriaxone), and/or aminoglycosides (gentamycin/amikacin), depending on the patient's condition.

Bacteria resistant or intermediate to both ciprofloxacin and cefotaxime were found in 72 cultures (54 %). Furthermore, of 71 tested cultures, 20 (28 % of these, 15 % of total) were also resistant to amikacin (figure 8).

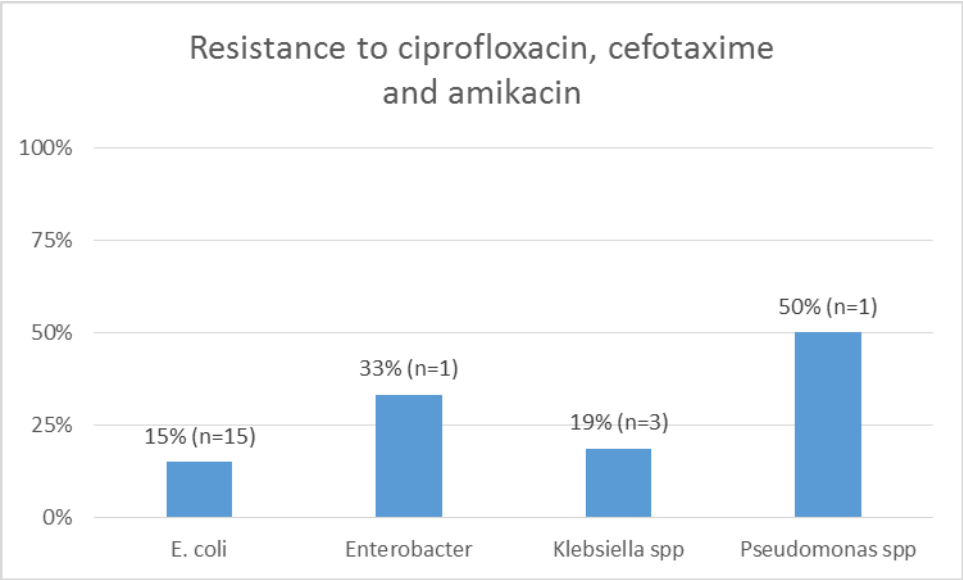

Figure 8. Resistance (as either resistant or intermediate) to ciprofloxacin, cefotaxime and amikacin by bacterial species. Percentage of total number per species, and number of cultures, is shown.

Of these, 10 were tested for imipenem (a carbapenem), which gave four resistant, one intermediate and five sensitive cultures. Of the five resistant or intermediate cultures, four were *E. coli* and one *Klebsiella spp*. Of these, four were tested for colistin and three for tigecykline – all of these were found to be sensitive.

Part 2

Patient characteristics

The bacteria found in Part 2 are presented in table 5.

Culture result	
	Number of cases
Acinetobacter spp	1 (2 %)
Citrobacter freundii	1 (2 %)
E. coli	36 (72 %)
Enterobacter	2 (4 %)
Klebsiella spp	5 (10 %)
Morganella morganii	1 (2 %)
Proteus spp	2 (4 %)
Pseudomonas spp	2 (4 %)

Table 5. Pathogens in Part 2.

There were 27 (54 %) cases were signs or symptoms of UTI were recorded. Information about clinical signs or clinical evaluation for the other 46 % of the patients was not mentioned in the medical records. Twenty cases (40 %) had an indwelling urinary catheter at the time the urine sample was taken, 23 % of female cases and 54 % of male cases. Cases with indwelling urinary catheter had a statistically significant increased likelihood of a non-*E. coli* pathogen (figure 9). No statistically significant differences in pathogens were seen between female and male cases, or in cases younger or older than 50 years.

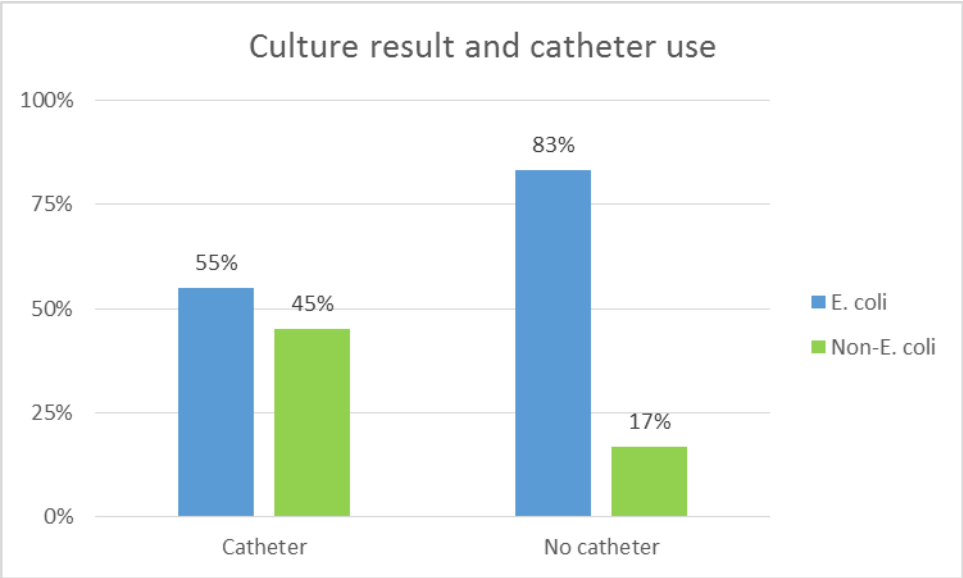

Figure 9. E. coli and non-E. coli pathogens by indwelling urinary catheter. Numbers are percentage of cases within each groups. P = 0.02, RR = 2.7, 95 % CI = 1.06 - 6.88.

Previous surgery or instrumentation of the urinary tracts had been done in 18 cases (36 %), five cases (10 %) had history of gynaecological surgery, 17 cases (34 %) had undergone any kind of surgery within the preceding three month and 24 cases (48 %) had been admitted to hospital within the preceding six month. Twenty-two cases (44 %) had been prescribed antibiotics within the preceding three months, 16 (73 %) of these cases had received two or more different classes of antibiotics (with a maximum of five classes). The different antibiotics prescribed and total number of occasions can be seen in table 6.

Previously prescribed antibiotics	
	Number of occasions
Amikacin	3
Azithromycin	1
Cefadroxil	1
Cefepin	1
Cefixime	2
Cefotaxime	2
Ceftriaxone	7
Chloramfenicol	1
Ciprofloxacin	5
Cloxacillin	4
Co-trimoxazole	2
Gentamycin	4
Levofloxacin	1
Metronidazol	4
Nitrofurantoin	1
Ofloxacin	11

Table 6. Antibiotics previously prescribed (within the preceding three month) and the total number of treatment episodes.

Nineteen cases (38 %) had been treated for UTI in the preceding 12 month, seven (37 %) of these cases more than once (two cases lacked record of number of previous UTI:s). Thirteen cases (26 %) had benign enlargement of prostate, five cases (10 %) had nephrolithiasis and one case (2 %) a bladder stone when the urine sample was taken.

The primary diagnosis is displayed in table 7, the associated pathogens in figure 10. The secondary diagnosis are shown in table 8.

Primary diagnosis			
	Number of cases		**Number of cases**
Infection; other	1	**Infection, total**	**14 (28 %)**
Infection; other, surgical, UTI	1		
Infection; other, UTI	1		
Infection; SSI	2		
Infection; surgical	3		
Infection; UTI	4		
Internal medicine condition	1	**Internal medicine condition, total**	**2 (4 %)**
Internal medicine condition – Surgical condition – Infection; other, UTI	1		
Surgical condition	9	**Surgical condition, total**	**12 (24 %)**
Surgical condition; trauma	2		
Urological condition	18	**Urological condition, total**	**22 (44 %)**
Urological condition; trauma	3		
Urological condition – Infection; UTI	1		
		Records not available	**3 (6 %)**

Table 7. Primary diagnosis. Total numbers can include more than one case, hence adding up to more than 100 %. Records not available - no record of primary diagnosis was found in medical chart.

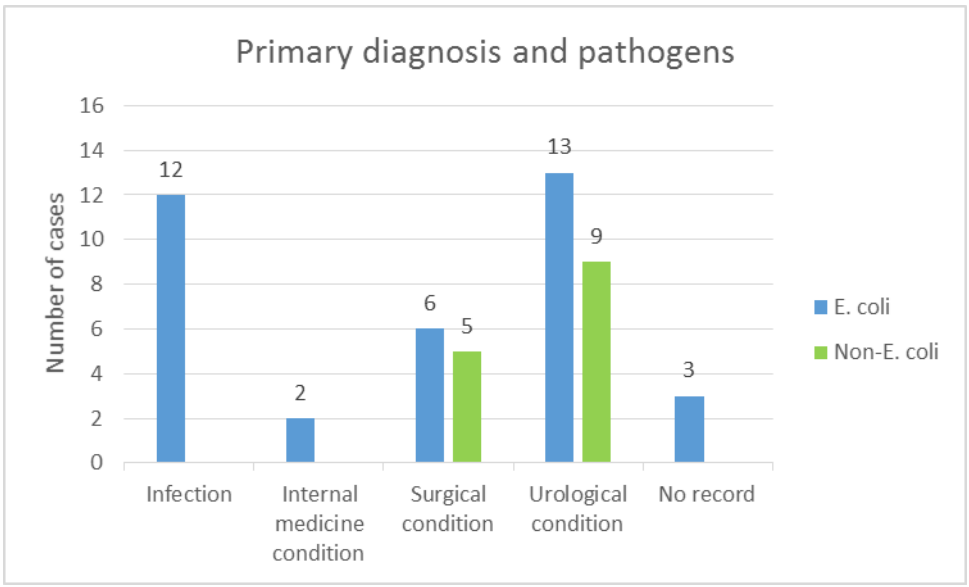

Figure 10. E. coli and non-E. coli pathogens in the main primary diagnoses (hence the different numbers compared to table 7). The differences between the pathogen groups were statistically significant for surgical- and urological condition vs infection and internal medicine condition (P = 0.001).

Secondary diagnosis	
	Number of cases, total
Cancer	1
Infection	2
Internal medicine condition	7
Orthopedic condition	1
Paralytic	4
Previous cancer	2
Psyciatric condition	1
Surgical condition	1
Urological condition	20

Table 8. Secondary diagnosis. NB! Some cases lacked record of secondary diagnosis, while other had more than one.

Treatment

To evaluate the antibiotic treatment of UTI and the rationality of the treatment proved difficult, mainly due to that many patients were treated for other conditions and infections than UTI, and to receive clarity on the indication(s) for each antibiotic was most often not possible. Also, only the date of the first dose was noted, and duration of each antibiotic was not included in the study. This made it difficult to determine if patients receiving more than one antibiotic were treated with multiple antibiotics simultaneously or if the antibiotic treatment was changed. Also, only new kinds of antibiotics to the patient were noted.

In total the patients were prescribed 87 antibiotics (table 9). In seven cases (14 %) there was no record of any antibiotic treatment at all. However: in 13 cases (26 %) three or more (with a maximum of six) different antibiotics were given.

All antibiotics prescribed	
	Number of occasions
Amikacin	9
Amoxicillin	1
Azithromycin	2
Cefazoline	1
Cefixime	2
Cefpodoxime	1
Ceftriaxone	6
Ciprofloxacin	14
Cloxacillin	5
Co-trimoxazole	3
Gentamycin	9
Metronidazol	7
Nitrofurantoin	5
Ofloxacin	22

Table 9. All antibiotics prescribed in the study.

The combinations used as first-line and second-line treatment is shown in table 10 and table 11, and the susceptibility to the antibiotics in first-line treatment in figure 11. First-line treatment were defined as antibiotics prescribed within five days before and seven days after culture sample was taken, second-line treatment as a new prescription of antibiotic after three days. Note however that "sensitive" only means that at least one antibiotic prescribed were effective against the bacteria in the culture, and it is not possible to say for how long time that antibiotic was used. It is worth noting however, that of the cases receiving antibiotic treatment, only 55 % of cases got at least one antibiotic to which the bacteria was sensitive. No statistical significant difference in treatment effectiveness (measured as resistant, not effective, and not tested vs sensitive) between cases in the surgical ward and cases in the surgical reference clinic could be seen.

First-line treatment	
	Number of occasions
Amikacin	3
Amikacin, Ofloxacin	2
Azitromycin	1
Cefazoline, Gentamycin	1
Cefexime	1
Cefpodoxime, Azithromycin, Amikacin	1
Ceftriaxone, Cefixime	1
Ceftriaxone, Ciprofloxacin, Metronidazol	1
Ceftriaxone, Cloxacillin, Amikacin	1
Ceftriaxone, Metronidazol, Amikacin	1
Ciprofloxacin	5
Ciprofloxacin, Gentamycin, Ofloxacin	1
Ciprofloxacin, Metronidazol	2
Ciprofloxacin, Ofloxacin	3
Cloxacillin	1
Cloxacillin, Metronidazol	1
Gentamycin	2
Gentamycin, Ofloxacin	2
Nitrofurantoin	2
Ofloxacin	9
Ofloxacin, Nitrofurantoin	1

Table 10. First-line treatments and number of occasions used. Defined as antibiotics prescribed within five days before and seven days after culture sample was taken.

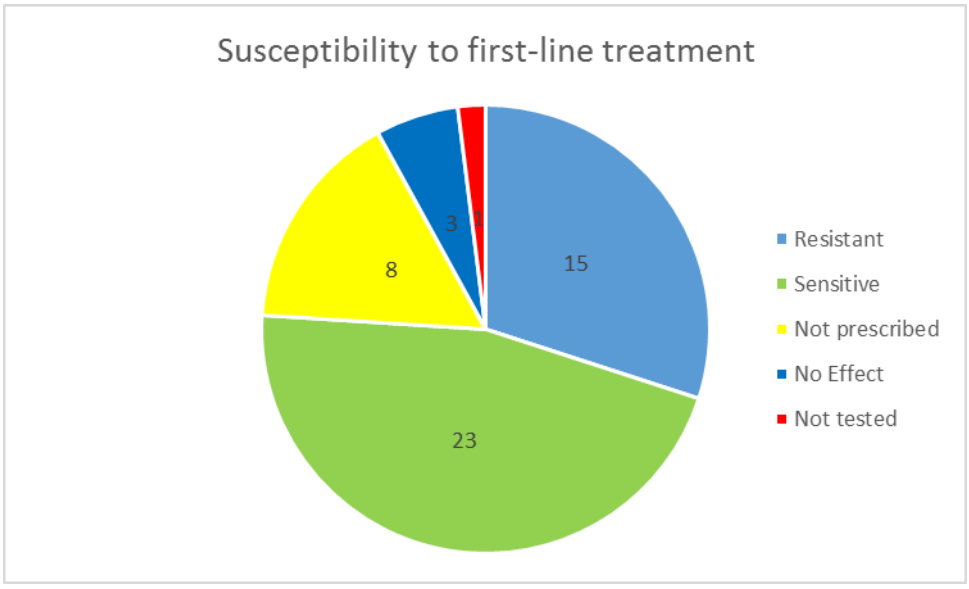

Figure 11. Susceptibility to any of the antibiotics prescribed as first-line treatment. Numbers are number of cases. Resistant - resistant to all antibiotics. Sensitive - sensitive to at least one antibiotic. Not prescribed - no antibiotic were prescribed in relation to the urine culture sample taken. No effect - all antibiotic(s) were of a class with no effect on the bacteria. Not tested - kind of antibiotic not tested for susceptibility.

Second-line treatment	
	Number of occasions
Amikacin (S), Co-trimoxazole (S)	1
Ciprofloxacin (R)	1
Co-trimoxazole (S)	2
Nitrofurantoin (S)	1
Ofloxacin (R), Nitrofurantoin (S)	1
Ofloxacin (S)	1

Table 11. Second-line treatment and number of occasions. Defined as a new prescription of antibiotic after three days.

Out of the 42 cases in table 10 (first-line treatment), 7 cases (17 %), received second-line treatment, which has been interpreted as treatment failure.

Antibiotic susceptibility in various risk factors

Finally, the antibiotic susceptibility to ciprofloxacin and cefotaxime were compared in different sub-groups in the study. Ciprofloxacin and cefotaxime were regarded as most relevant antibiotics to compare since they were the most common empiric treatment for UTI (amikacin excluded due to low overall resistance rate). Intermediate cultures have been considered resistant in the statistical calculations.

In Part 1, a statistical significant higher resistant to both ciprofloxacin and cefotaxime were found in cultures from the surgical ward compared to cultures from the surgical reference clinic (figure 12). In Part 2, statistical significant differences were found for resistance to ciprofloxacin (but not to cefotaxime) between cases with or without indwelling urinary catheter (figure 13), and in cases with previous admittance to hospital (figure 14). No statistically significant increase in resistance to either ciprofloxacin or cefotaxime was found in cases with previous surgery of the urinary tract, urological condition as primary diagnosis, previous antibiotic treatment, and previous UTI.

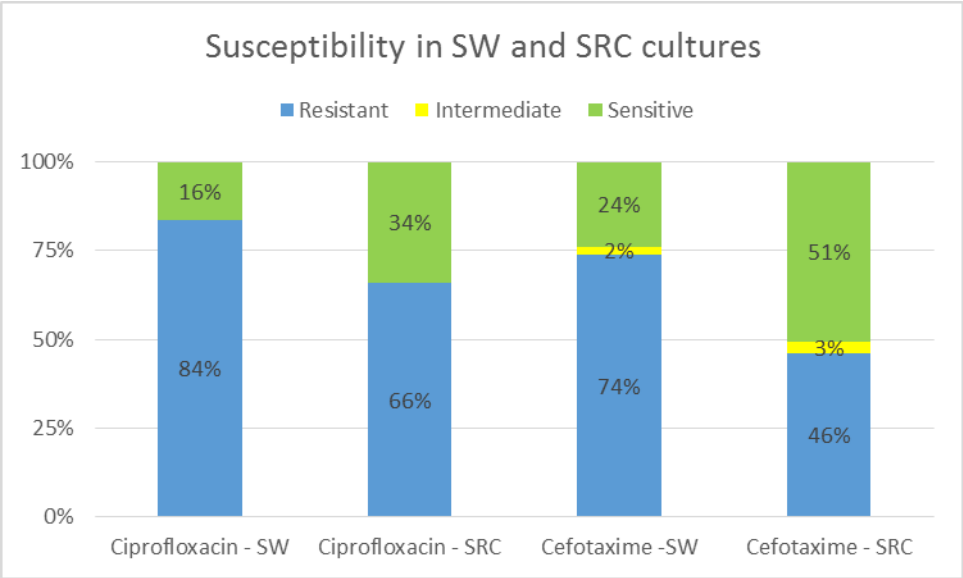

Figure 12. Susceptibility to ciprofloxacin and cefotaxime in cultures from the surgical ward (SW) and the surgical reference clinic (SRC). Numbers are percentage of cases within each groups. Ciprofloxacin: P = 0.02, RR = 1.27, 95 % CI = 1.04 - 1.55; Cefotaxime: P = 0.002, RR = 1.54, 95 % CI = 1.18 - 2.01

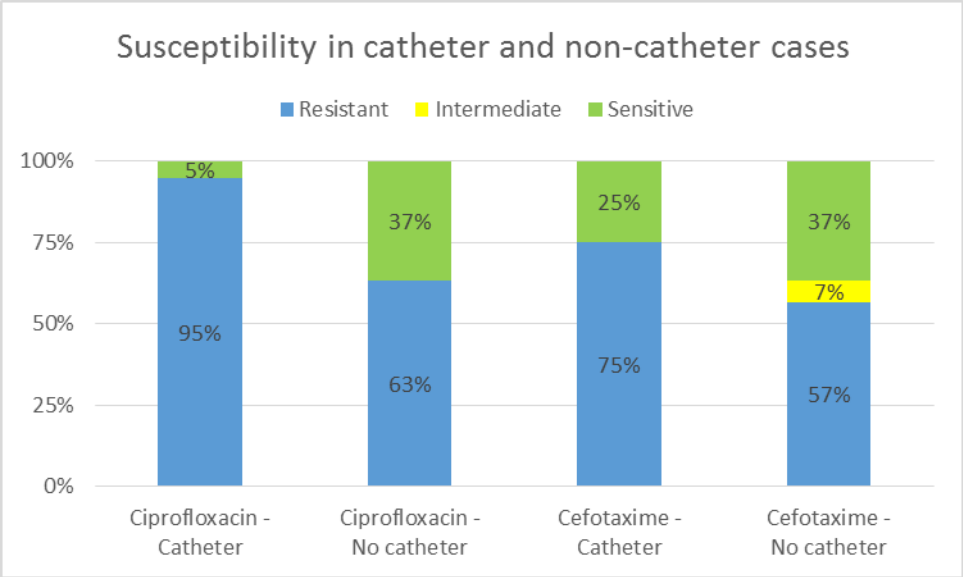

Figure 13. Susceptibility to ciprofloxacin and cefotaxime by indwelling urinary catheter. Numbers are percentage of cases within each groups. Ciprofloxacin: P = 0.005, RR = 1.5, CI = 1.12 - 2.01; Cefotaxime: P = 0.21, RR = 1.18, 95 % CI = 0.82 - 1.72

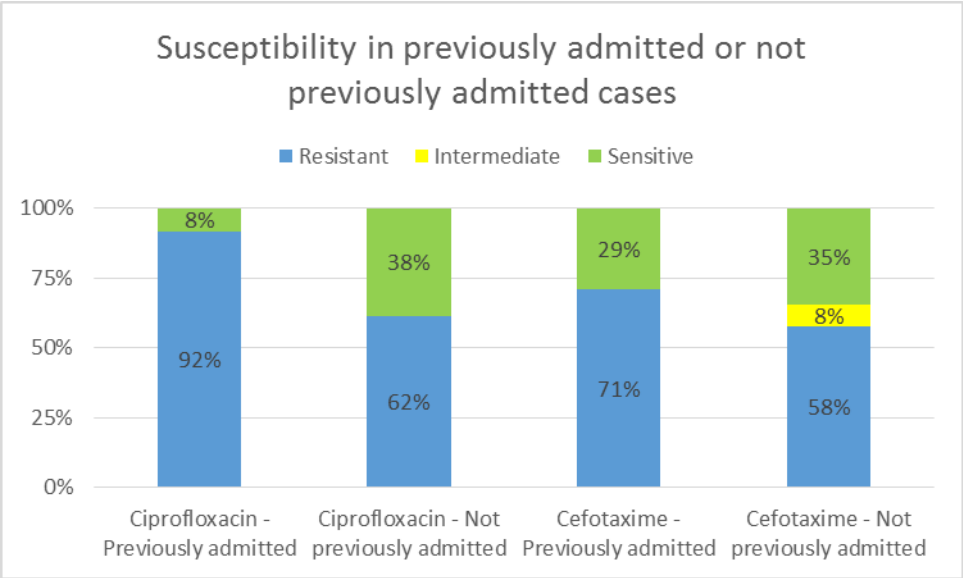

Figure 14. Susceptibility to ciprofloxacin and cefotaxime in cases by previously admittance within the preceding six month. Numbers are percentage of cases within each groups. Ciprofloxacin: P = 0.008, RR = 1.49, CI = 1.07 - 2.07; Cefotaxime: P = 0.35, RR = 1.08, 95 % CI = 0.74 - 1.58

DISCUSSION

The main finding in this study were the exceptionally high levels of resistance to virtually all clinically relevant antibiotics. Highest degrees of resistance were found in fluoroquinolones, the most commonly prescribed antibiotics in this study. Amikacin and nitrofurantoin proved to be the antibiotics with the most favourable resistance rates (nitrofurantoin only for *E. coli*). Although carbapenems were tested in a too limited number of cultures to draw reliable conclusions, 50 % were resistant. In the light of that previous studies in Nepal have found no resistance to carbapenems, this raises a warning that resistance to carbapenems might be on the rise in Nepal. Throughout monitoring of local antibiotic resistance is of paramount importance to tackle this problem.

The other main finding was the ineffectiveness of the first line treatments. Only 55 % of cases treated with antibiotics were treated with at least one antibiotic that were effective against the bacteria. In many cases, the effective antibiotics were only aminoglycosides. The high rates of

resistance compose a formidable challenge to effective empirical treatment. This finding emphasizes the need of local and national clinical guidelines for empirical treatment as well as the monitoring of bacterial cultures in postoperative infections.

Looking into the differences in susceptibility to ciprofloxacin and cefotaxime for a number of known risk factors for complicated UTI and ABR, significantly higher resistance were found in cultures from the surgical ward compared to the surgical reference clinic. In cases with indwelling urinary catheter and in cases with previous admittance to hospital, a statistically significant higher resistance to ciprofloxacin were found. This could probably be explained by a higher risk of (previously) being colonized by a strain of fluoroquinolone-resistant bacteria in an environment with a high presence of resistant bacteria. The lack of statistical significant differences between the other groups could be explained by the small number of cases in each group, or confounding factors.

Part 1

Although high levels of ABR were found for all pathogens to all antibiotics, especially worrying are the high rates of resistance against fluoroquinolones, which besides being the most used antibiotics in this study, also are a per oral (p.o.) antibiotic meaning it can be used in an out-patient settings. Co-trimoxazole – another p.o. drug – had in *E. coli* isolates a slightly lower resistance rate than fluoroquinolones. Nitrofurantoin (p.o), had a relatively low resistance rate in *E. coli* (24 % resistant, 13 % intermediate), but high for both *Klebsiella spp* and non-*E. coli* (over 80 % resistant). Third generation cephalosporins (cefotaxime/ceftriaxone, both intravenously [i.v.] administrated) had lower resistance rates than fluoroquinolones, although still high, and a pronounced difference between *E. coli*, *Klebsiella spp* and non-*E. coli* were noted. No testing for ESBL-producing bacteria were

conducted, but a study from Kathmandu in 2013 found 13.51 % of *E. coli* and 16.55 % of *Klebsiella pneumoniae* to be ESBL positive [37]. Amikacin (i.v.) had the lowest rates of resistance among all the commonly used antibiotics, and noticeably lower than gentamycin. No major differences were seen between *E. coli*, *Klebsiella spp* and non-*E.coli*.

Carbapenems, piperacillin-tazobactam, colistin and tigecycline were only tested on a small number of isolates, and hence reliable conclusions cannot be made. However, colistin and tigecyclin seem to have low rates of resistance. On the other hand, the resistance to carbapenems appear to be around 50 %, a finding contradictory to other studies where no resistance to carbapenems have been found [1, 37]. This indicates a possible rise in resistance to carbapenems, and once again stresses the importance of ABR monitoring. No use of carbapenems, piperacillin-tazobactam, colistin or tigecycline was found in the studied medical records. This raises the question on the origin of the resistance to carbapenems found in this study. A study from New Delhi in 2011 found NDM-1-producing bacteria in drinking water and seepage samples, indicating a possibility of acquiring resistant bacteria outside of health-care facilities, and this might be a possible origin, apart from other departments or health-care facilities [44].

Compared to previous studies from Nepal [36-38] and data from WHO [1], the susceptibility patterns were similar, although resistance rates were often higher in this study. In these studies the resistance of *E. coli* to fluoroquinolones were 35.9 % - 64.3 %, and to third generation cephalosporins 19.2 % - 37.9 %. In *Klebsiella spp* the resistance to fluoroquinolones were 4.3 % - 16.7 %, and to third generation cephalosporins 3.2 % - 48.3 %. However, different cephalosporins (ceftazidime, cefotaxime and ceftriaxone) were used in the different studies, making comparisons of resistance to cephalosporins somewhat uncertain.

Nitrofurantoin had a comparable resistance rate in [38], and a lower (7.9 %) in [36]. Amikacin were the antibiotic with highest susceptibility in all three studies [36-38].

The findings in this study of high levels of ABR, especially considered together with the widespread use of fluoroquinolones and the low success rate of first line treatments, a change in empirical treatment of UTI in surgical patients at Patan Hospital could be advised. Fluoroquinolones and third generation cephalosporins should be avoided, both due to low probability of treatment success, and also to slow down even further increase in resistance. A high resistance to these antibiotics might increase the use of carbapenems, and with it the risk of increased resistance to them. As a p.o. treatment, nitrofurantoin could be used as a first line treatment in patients (including men) with uncomplicated, lower UTI without fever and with high probability of *E. coli* [13, 36, 37, 45]. Considering that the resistance to co-trimoxazole were similar to that of fluoroquinolones and third generation cephalosporins in *E. coli* isolates, it too could be considered as an alternative. When an i.v. drug is needed, amikacin seem to be the best alternative, with good effect on upper UTI and septicaemia and high susceptibility among all major pathogens [36, 37]. Nitrofurantoin and amikacin are also effective in cases of ESBL-producing pathogens [37, 45].

Being a highly selected group of patients with a high prevalence of risk factors for increased ABR, conclusions and extrapolations to the general patient population should be done with cautions. However, a general shift in treatment of UTI towards nitrofurantoin and amikacin could be advised. The importance of culture and antibiotic susceptibility testing cannot be overstated. Since Patan Hospital accepts patients from all over the country, a widespread ABR in the hospital also implies the risk of people from remote areas acquiring resistant bacteria, and then may act as carriers of drug resistant pathogens back to their native areas.

In this study, a lesser percentages of cultures were positive compared to previous studies from Nepal. A study from Kathmandu had 30.8 % positive cultures in a hospital setting with a mixed study population of in- and out-patients [36], whereas a study from Pokhara found 71.1 % positive cultures in hospitalized patients only [38]. It was in this study not possible to know if the patient was already on any kind of antibiotic, and whether this could be a contributing factor to the relatively low percentage of positive cultures. However, while there could be a number of other reasons contributing to the different figures, it shows the importance of culture in addition to patient history and clinical examination to confirm the diagnosis of UTI.

The dominating bacteria found *E. coli*. In cultures with more than 100 000 CFU/ml *E. coli* made up 75 % of positive cultures, and in cultures from the surgical ward only the number were 69 %. This can be compared to 81.3 % in [36], and 59.4 % in [38]. In a North American and European material, *E. coli* made up 47 % and 36 % of positive cultures among hospitalized patients, respectively [46]. Among out-patients, *E. coli* often make up over 80 % of UTI [13]. This corresponds well with the conception that the proportion of complicated UTI caused by non-*E. coli* pathogens is higher in a hospital setting. Still, the proportion of *E. coli* in hospitalized patients in this study was relatively high.

Part 2

The success rate of the first line treatment reflects the high resistance rate. Only 55 % of cases treated with antibiotics were treated with at least one antibiotic that were effective against the bacteria. In many cases, the only effective antibiotic were an aminoglycoside, and since this is an i.v. drug and hence only can be administrated to admitted patients, the real effectiveness of the antibiotic treatment once the patient have been discharged is hard to determine and probably even somewhat lower than suggested. 17 % of cases had treatment failure and

received second-line treatment. This figure is however very uncertain since only antibiotics prescribed by PH are included, and if the patient sought medical attention elsewhere, or bought antibiotics over-the-counter, this is not included.

Even though there are considerable room for improvement, improving the effectiveness of the antibiotic treatment is a daunting task in the light of the high rates of ABR. Clinical guidelines could play an important role in this work. As discussed above, an increased use of nitrofurantoin and amikacin could be advised. Empirical antibiotic treatment should be kept to a minimum, and if the patient's condition allowing, antibiotic treatment could in certain circumstances await the culture result. Also, especially in patients with indwelling urinary catheter, antibiotic treatment should only be done when an actual UTI is suspected, and not only to treat a positive urine culture (i.e. ABU).

The most common antibiotics used in this study – both as first-line treatment, in overall treatment and previously prescribed – were fluoroquinolones, ceftriaxone and aminoglycosides (amikacin and gentamycin equally used), which is consistent with reports on empirical treatment in PH [43]. Co-trimoxazole and nitrofurantoin were used only sporadically. To facilitate future studies on antibiotic prescription, a more systematic and standardized way of documentation should be implemented. This does not necessarily only be a part of the patient's medical chart, but could be recorded for the whole ward/clinic together, and contain information as e.g. antibiotic prescription, indication, culture result etc.

A peculiar finding is that in only 54 % of the cases signs and/or symptoms of UTI were noted in the medical chart. It is, however, hard to determine if the other 46 % of the cases actually had ABU, or if information in the charts simply were missing in some cases. To facilitate

future studies, a more meticulous and/or standardized recording of key information is recommended.

A majority of the patients were men, contradictory to other studies [32, 36-38]. Females dominated in the younger ages and males in the older. Patients with some kind of urological condition as primary diagnosis dominated greatly, with surgical conditions and infections in a shared second place. Urological condition was also by far the most common secondary diagnosis. 40 % of the cases had an indwelling urinary catheter when the urine sample were taken, a majority of them being males. Use of indwelling urinary catheter, and surgical- and urological condition were associated with a statistically significant increased likelihood of non-*E. coli* pathogens. A large number of the cases had one or several risk factors for (complicated) UTI and/or ABR, such as previous instrumentation in the urinary tracts, previous surgery, previous admittance to hospital, previous UTI, and (multiple) previous antibiotics treatments.

Study limitations

The bacterial cultures in this study came from a selected group of patients with an overrepresentation of risk factors for complicated UTI and ABR, and the findings in this study are not applicable to community-acquired, uncomplicated UTI and UTI-patients in general. It is more likely that the pathogens found and their resistance patterns are representative of the bacteria circulating in the hospital than in the community at large.

Both the log books and the medical charts were hand written. Even though the utmost care have been taken, misinterpretations cannot be ruled out due to the handwritten data and the language barrier. The medical charts were paper sheets stacked within a folder and there is a

possibility that sheets were missing. Also, the records are doctor-dependent and absence of information could be interpreted both as lack of recording or e.g. absence of symptoms.

The inclusion criteria of over 100 000 CFU/ml is not used everywhere for UTI, e.g. the Swedish criteria for UTI differ (1, 6). It was in this study not possible to know if the patient was already on any kind of antibiotic, and whether this could be a contributing factor to the relatively low percentage of positive cultures.

All cases in Part 2 had positive urine cultures, hence there is no control group to compare with. The dropout rate was high, reasons for that could be that the medical charts was misplaced or that the number recorded was incorrectly or unclear. Not all cases had follow-up to evaluate treatment success. This made conclusions about outcome were hard to draw. Also, since only the medical charts from Patan Hospital were available, it is not possible to know if the patients were treated at other health-care facilities, or bought antibiotics over-the-counter.

Information about the antibiotics prescribed was limited to type of antibiotic and first day of prescription, and not duration, dose, or re-prescription. Hence, conclusions about the treatment are somewhat uncertain. Many patients had other infections and conditions requiring antibiotics, and the indications for each antibiotic were most often not possible to determine.

CONCLUSIONS

The findings in this study show an exceptionally high rate of ABR in the surgical department of Patan Hospital, and a low rate of effective empirical antibiotic treatment. To tackle this problem both national and local guidelines are needed to ensure effective (empirical) treatment and to hold back an even further increase in ABR. Empirical treatment should be minimized, and an increased use of nitrofurantoin and amikacin is recommended since these two drugs showed the lowest resistance rates (nitrofurantoin in *E. coli*, amikacin in all pathogens). Since rates of ABR can vary between settings and over time, a throughout local surveillance of ABR can play an important role in helping clinicians choose the optimal empiric antibiotic treatment and also be an important tool to hamper further increase of ABR. Further studies are needed in other departments, hospitals and out-patients settings. Further studies are also needed on outcome of antibiotic treatment, and to determine if – and to what degree – ABR is a threat to patient wellbeing and survival.

POPULÄRVETENSKAPLIG SAMMANFATTNING PÅ SVENSKA

Urinvägsinfektioner är en av de vanligaste infektionerna hos människor. Det är också en mycket vanlig orsak till förskrivning av antibiotika. På grund av detta spelar urinvägsinfektioner en stor roll för uppkomst och spridning av bakterier som är okänsliga för antibiotika. Okänsliga bakterier kan göra infektioner svårbehandlade med de antibiotika som vanligen används. Denna studie undersökte urinvägsinfektioner på en kirurgklinik på ett sjukhus i utkanten av Kathmandu i Nepal. Bland de bakterier som hittades var en hög andel okänsliga mot de antibiotika som vanligen användes. Det kan bero på att många patienter hade flera olika riskfaktorer för att få en infektion med en okänslig bakterie. Endast 55 % av behandlade patienter fick i första behandlingsomgången en antibiotika som var effektiv.

Patienter som var inneliggande på avdelning, hade urinkateter eller tidigare hade varit inlagda på sjukhuset hade högre risk att vara infekterade med en okänslig bakterie. Utifrån fynden i denna studie kan det rekommenderas att utföra en bakterieodling innan behandling, att om möjligt avvakta med antibiotikabehandling tills odlingssvar föreligger, samt att öka användningen av vissa antibiotika. Kunskap om andelen okänsliga bakterier är viktigt och kan hjälpa läkare att välja rätt antibiotika. Rätt antibiotika kan göra behandlingen effektivare och bromsa uppkomsten av okänsliga bakterier. Fler studier där man undersöker okänsliga bakterier på andra avdelningar, på andra sjukhus och inte minst i öppenvården behövs.

ACKNOWLEDGEMENT

The author is very thankful for the guidance and support from my supervisors Dr Pukar Maskey, Dr Amit Arjyal and Dr Poojan Shrestha during the stay at Patan Hospital. Macha Bhai Shakya, Manish Maharjan and Aswin KC are all thanked for their support in practical matters and especially for the assistance in finding the patient charts, and Dr Leif Dotevall for his patience and keen help during the process of analysing and writing.

REFERENCES

1. WHO, *Antimicrobial resistance: global report on surveillance*. 2014.
2. Laxminarayan, R., et al., *Antibiotic resistance-the need for global solutions.* Lancet Infect Dis, 2013. **13**(12): p. 1057-98.
3. *Vital signs: carbapenem-resistant Enterobacteriaceae.* MMWR Morb Mortal Wkly Rep, 2013. **62**(9): p. 165-70.
4. Elemam, A., J. Rahimian, and W. Mandell, *Infection with panresistant Klebsiella pneumoniae: a report of 2 cases and a brief review of the literature.* Clin Infect Dis, 2009. **49**(2): p. 271-4.
5. Sjolund, M., et al., *Dissemination of multidrug-resistant bacteria into the Arctic.* Emerg Infect Dis, 2008. **14**(1): p. 70-2.
6. Pitout, J.D. and K.B. Laupland, *Extended-spectrum beta-lactamase-producing Enterobacteriaceae: an emerging public-health concern.* Lancet Infect Dis, 2008. **8**(3): p. 159-66.
7. Queenan, A.M. and K. Bush, *Carbapenemases: the versatile beta-lactamases.* Clin Microbiol Rev, 2007. **20**(3): p. 440-58.
8. Yong, D., et al., *Characterization of a new metallo-beta-lactamase gene, bla(NDM-1), and a novel erythromycin esterase gene carried on a unique genetic structure in Klebsiella pneumoniae sequence type 14 from India.* Antimicrob Agents Chemother, 2009. **53**(12): p. 5046-54.
9. Nordmann, P., T. Naas, and L. Poirel, *Global spread of Carbapenemase-producing Enterobacteriaceae.* Emerg Infect Dis, 2011. **17**(10): p. 1791-8.
10. Nordmann, P., et al., *Does broad-spectrum beta-lactam resistance due to NDM-1 herald the end of the antibiotic era for treatment of infections caused by Gram-negative bacteria?* J Antimicrob Chemother, 2011. **66**(4): p. 689-92.
11. Struelens, M.J., et al., *New Delhi metallo-beta-lactamase 1-producing Enterobacteriaceae: emergence and response in Europe.* Euro surveillance : bulletin Européen sur les maladies transmissibles., 2010. **15**(46).

12. Hawser, S.P., et al., *Emergence of high levels of extended-spectrum-beta-lactamase-producing gram-negative bacilli in the Asia-Pacific region: data from the Study for Monitoring Antimicrobial Resistance Trends (SMART) program, 2007.* Antimicrob Agents Chemother, 2009. **53**(8): p. 3280-4.
13. Sandberg, T., S. Hansson, and J. Nyman, *Urinvägsinfektioner*, in *Läkemedelsboken 2014*, H. Ramström, Editor., Läkemedelsverket: Uppsala.
14. O'Brien, T.F., *Emergence, spread, and environmental effect of antimicrobial resistance: how use of an antimicrobial anywhere can increase resistance to any antimicrobial anywhere else.* Clin Infect Dis, 2002. **34 Suppl 3**: p. S78-84.
15. Allegranzi, B., et al., *Burden of endemic health-care-associated infection in developing countries: systematic review and meta-analysis.* The Lancet, 2011. **377**(9761): p. 228-241.
16. ECDC. *Annual epidemiological report on communicable diseases in Europe 2008: report on the state of communicable diseases in the EU and EEA/EFTA countries [Internet]*. 2015-07-08]; Available from: http://www.ecdc.europa.eu/en/publications/Publications/0812_SUR_Annual_Epidemiological_Report_2008.pdf.
17. Goossens, H., et al., *Outpatient antibiotic use in Europe and association with resistance: a cross-national database study.* Lancet, 2005. **365**(9459): p. 579-87.
18. Morgan, D.J., et al., *Non-prescription antimicrobial use worldwide: a systematic review.* Lancet Infect Dis, 2011. **11**(9): p. 692-701.
19. Dromigny, J.A., et al., *Risk factors for antibiotic-resistant Escherichia coli isolated from community-acquired urinary tract infections in Dakar, Senegal.* J Antimicrob Chemother, 2005. **56**(1): p. 236-9.
20. Apisarnthanarak, A. and L.M. Mundy, *Correlation of antibiotic use and antimicrobial resistance in Pratumthani, Thailand, 2000 to 2006.* Am J Infect Control, 2008. **36**(9): p. 681-2.

21. Johansson EW, C.L., Newby H, Young M, Wardlaw T., *Pneumonia and diarrhoea: tackling the deadliest diseases for the world's poorest children.* 2012, UNICEF: New York.
22. Baqui, A.H., et al., *Zinc therapy for diarrhoea increased the use of oral rehydration therapy and reduced the use of antibiotics in Bangladeshi children.* J Health Popul Nutr, 2004. **22**(4): p. 440-2.
23. ECDC/EMEA. *Technical Report. The bacterial challenge: time to react.* 2009 2015-07-08]; Available from: http://ecdc.europa.eu/en/publications/Publications/0909_TER_The_Bacterial_Challenge_Time_to_React.pdf.
24. Liu, L., et al., *Global, regional, and national causes of child mortality: an updated systematic analysis for 2010 with time trends since 2000.* Lancet, 2012. **379**(9832): p. 2151-61.
25. Byarugaba, D.K., *A view on antimicrobial resistance in developing countries and responsible risk factors.* Int J Antimicrob Agents, 2004. **24**(2): p. 105-10.
26. Paterson, D.L., *The role of antimicrobial management programs in optimizing antibiotic prescribing within hospitals.* Clin Infect Dis, 2006. **42 Suppl 2**: p. S90-5.
27. Davey, P., et al., *Interventions to improve antibiotic prescribing practices for hospital inpatients.* Cochrane Database Syst Rev, 2013. **4**: p. CD003543.
28. Rahal, J.J., et al., *Class restriction of cephalosporin use to control total cephalosporin resistance in nosocomial Klebsiella.* JAMA, 1998. **280**(14): p. 1233-7.
29. Bergstrom, R., *The role of the pharmaceutical industry in meeting the public health threat of antibacterial resistance.* Drug Resist Updat, 2011. **14**(2): p. 77-8.
30. Fernebro, J., *Fighting bacterial infections-future treatment options.* Drug Resist Updat, 2011. **14**(2): p. 125-39.
31. Gastmeier, P., et al., *Prevalence of nosocomial infections in representative German hospitals.* J Hosp Infect, 1998. **38**(1): p. 37-49.

32. Rané, A., R. Dasgupta, and SpringerLink, *Urinary Tract Infection: Clinical Perspectives on Urinary Tract Infection / [Elektronisk resurs]*. 2013, London: Springer London.
33. Townsend, C.M. and D.C. Sabiston, *Sabiston textbook of surgery: the biological basis of modern surgical practice*. 2008, Philadelphia, Pa: Elsevier Saunders.
34. *Ne.se [Internet]*. 2014-11- 08]; Available from: http://www.ne.se.ezproxy.ub.gu.se/uppslagsverk/encyklopedi/l%C3%A5ng/nepal.
35. *Worldbank.org [Internet]*. 2014-11-08]; Available from: http://www.worldbank.org/en/country/nepal/overview.
36. Baral, P., et al., *High prevalence of multidrug resistance in bacterial uropathogens from Kathmandu, Nepal.* BMC Res Notes, 2012. **5**: p. 38.
37. Chander, A. and C.D. Shrestha, *Prevalence of extended spectrum beta lactamase producing Escherichia coli and Klebsiella pneumoniae urinary isolates in a tertiary care hospital in Kathmandu, Nepal.* BMC Res Notes, 2013. **6**: p. 487.
38. Das, R.N., et al., *Frequency and susceptibility profile of pathogens causing urinary tract infections at a tertiary care hospital in western Nepal.* Singapore Med J, 2006. **47**(4): p. 281-5.
39. *Pahs.edu.np [Internet]*. 2014-11-08]; Available from: http://www.pahs.edu.np/.
40. Dean, A., K. Sullivan, and M. Soe. *OpenEpi: Open Source Epidemiologic Statistics for Public Health [Internet]*. 2014-11-27]; Available from: http://www.openepi.com.
41. Pandey, S., S. Raza, and C.P. Bhatta, *The aetiology of the bloodstream infections in the patients who presented to a tertiary care teaching hospital in kathmandu, Nepal.* J Clin Diagn Res, 2013. **7**(4): p. 638-41.
42. Murdoch, D.R., et al., *The etiology of febrile illness in adults presenting to Patan hospital in Kathmandu, Nepal.* Am J Trop Med Hyg, 2004. **70**(6): p. 670-5.
43. Maskey, P., *E-mail correspondence 2014-05-23*.

44. Walsh, T.R., et al., *Dissemination of NDM-1 positive bacteria in the New Delhi environment and its implications for human health: an environmental point prevalence study.* Lancet Infect Dis, 2011. **11**(5): p. 355-62.
45. Tasbakan, M.I., et al., *Nitrofurantoin in the treatment of extended-spectrum beta-lactamase-producing Escherichia coli-related lower urinary tract infection.* Int J Antimicrob Agents, 2012. **40**(6): p. 554-6.
46. Wagenlehner, F.M.E. and K.G. Naber, *Antibiotics and Resistance of Uropathogens.* EAU Update Series, 2004. **2**(3): p. 125-135.

APPENDIX A

IRC-PAHS

InstitutionalReviewCommittee -PatanAcademy of Health Sciences

पाटन स्वास्थ्य विज्ञान प्रतिष्ठान

Lagankhel-5, Lalitpur, Nepal लगनखेल- ५, ललितपुर, नेपाल

Ref: std1402141050
Date: 2014-02-14

To:
Adam Oscarson,

Thank you for submission of your research proposal. This is to inform that your proposal has been approved by "IRC-PAHS". We are confident that you will follow the guidelines, and provide necessary information/materials as and when required by the "IRC-PAHS". For any queries please contact IRC-PAHS secretary/chairman.

Title of study: "Study of antibiotic resistance in a surgical ward at Patan Hospital, Nepal"

Principle Investigator: Adam Oscarson, University of Gothenburg, Sweden.

Sincerely,

Assoc. Prof. Dr. Nabees Man Singh Pradhan
Member Secretary, IRC-PAHS
Patan Academy of Health Sciences (PAHS), Ktm, Nepal
Phone:977-1-5521034, Fax: 977-1-5548008, Cell: 977-1-9851094037
irc-pahs@pahs.edu.np,nabeesman@gmail.com