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FACULTY OF SCIENCES
Master of Statistics

Master's thesis

Antimicrobial activity of nitrofurantoin against multidrug-resistant urinary *Escherichia coli* from Belgian outpatients

Supervisor :
Prof. dr. Niel HENS

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Prof.dr. SAMUEL COENEN

Glory Abong Atud

Thesis presented in fulfillment of the requirements for the degree of Master of Statistics

Transnational University Limburg is a unique collaboration of two universities in two countries: the University of Hasselt and Maastricht University.

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Abstract

Background: Urinary tract infections (UTIs) are commonly caused by *E.coli*, are a frequent reason for consulting in primary health care and remain one of the most common indications for antibiotic prescribing. Antimicrobial resistance is major health problem.

Objectives: To analyse antimicrobial susceptibility testing (AST) data and describe the prevalence of multidrug-resistant(MDR) *E. coli* amongst Belgian outpatients, to assess the susceptibility to oral antibiotics commonly used to treat UTI in MDR *E. coli* and to assess the effect of age, gender, amount of antibiotic and number of antibiotic classes taken by the patient before urine sample collection on MDR *E.coli*.

Method: AST data, patient characteristics of reimbursed antibiotic therapy during the period of January to December 2005 from the IARG study were analysed. Isolates for susceptibility to ampicillin, cefalothin, ciprofloxacin, nitrofurantoin, trimethoprim/sulfamethoxazole and amoxicillin/clavulanate, representing six separate antibiotic classes, were eligible for analysis. Isolates were classified as resistant to one up to a maximum of six of the antibiotics considered. Multidrug resistance was defined as resistance of an *E. coli* isolate to at least three of six antibiotics. Tests of difference in proportions were used to determine the difference in susceptibility to the six antibiotics in MDR isolates.
The effect of age, gender, amount of antibiotic and number of antibiotic classes taken by the patient before urine sample collection on MDR was assessed via a logistic regression.

Results: 30.04 % of the 6478 *E.coli* isolates considered for analysis were MDR. Amongst the isolates that showed resistance to three, four or five antibiotics, 4.14%, 15.77% and 25.00% were resistant to nitrofurantoin.
Wide-spread resistance was seen with ampicillin (97.31%, 98.71% and 100%), amoxicillin/clavulanate (35.20%, 52.21% and 84.38%), ciprofloxacin (24.74%, 60.57% and 96.18%), trimethoprim/sulfamethazole (51.24%, 78.71% and 94.79%) and cefalothin (87.37%, 94.01% and 99.65%) for isolates resistant to three, four or five antibiotics, respectively.
The proportion of isolates non-susceptible to nitrofurantoin was significantly lower (p value <0.0001) compared to the other five antibiotics regardless of whether intermediate AST results were considered as susceptible or non-susceptible. The risk of MDR *E. coli* isolate increases with increase in the number of antibiotic classes(23%) and with

a ten fold increase in the Daily Defined Dose (4%) taken by patient before urine sample collection. The risk of MDR *E. coli* was higher(17%) for males compared to females, and for patients at least sixty years (27%) compared to patients younger than sixty.

Conclusion: Nitrofurantoin preserves its antimicrobial activity against MDR *E. coli*, thus it remains a reliable first-line empirical treatment for acute uncomplicated cystitis. Consumption of a large amount of antibiotics and different subgroups of antibiotics is a risk for MDR *E. coli*. It is more likely to isolate MDR *E. coli* from males compared to females and from patients at least sixty compared to patients younger than sixty years .

Dedication

To the memory of my son Ryan-Kyle.

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1 Introduction

Urinary tract infections (UTI) are amongst the most prevailing infectious diseases with a substantial financial burden on society. Gram-negative bacteria are the most relevant bacteria detected from patients with UTI, with more than 50% of which is accounted for by *E. coli* (Blomgran et al, 2004).

In the USA, UTI are responsible for over 7 million physician visits annually (Foxman, 2002) and about 15% of all community-prescribed antibiotics in the USA are dispensed for UTI (Mazzulli, 2002), data from some European countries suggest a similar rate (UVI, 2007). The causative pathogen in 75 - 95% of UTI cases is *E. coli*, and the majority of these infections occur among outpatients (Sanchez et al, 2013).

Antibiotics are one of the most important therapeutic discoveries in medical history. They have changed the way bacterial infections are treated and have contributed to reduce the mortality and morbidity from bacterial diseases. As a result, they are commonly used and thus liable to misuse. Antibiotics are often unnecessarily prescribed for viral infections, against which they have no effect, especially broad-spectrum antibiotics when diagnoses are not accurately made.

Misuse of antibiotics has led to the emergence and selection of resistant bacteria. Doctors in Europe and worldwide sometimes face situations where infected patients cannot be treated adequately because the responsible bacteria are completely resistant to available antibiotics (ECDC Fact Sheet). Thus antimicrobial resistance is a major public health problem (Levy and Marshall, 2004).

Resistance to multiple drugs was first detected amongst enteric bacteria (*E. coli*, *Shigella* and *Salmonella*) in the late 1950s to early 1960s (Watanabe, 1963). The past two decades have witnessed major increases in emergence and spread of multidrug-resistant (MDR) bacteria and increasing resistance to broad-spectrum antibiotics, such as fluoroquinolones and certain cephalosporins (Levy et al, 2004); due to an over consumption of these two groups and the parallel development of co-resistance to other antibiotics

(Cassier et al, 2011). An urgent and strong grip on this threatening development is thus required. With only a few new antibiotics expected in the coming 5 to 10 years, prudent use of available antibiotics is the only option to delay the development of resistance (Gyssens, 2011) and the medical community has a responsibility to participate in this combat.

Formerly infections with MDR *E. coli*, also known as ESBL (extended spectrum beta-lactamase), were assumed to be a hospital phenomenon. However, according to a recent presentation at the Inter science Conference on Antimicrobial agents and Chemotherapy (ICAAC) showed that ESBL are now also present amongst outpatients. This presentation was on surveyed records from five hospitals across the USA (New York, Pennsylvania, Michigan, Texas, and Iowa) which showed that amongst the identified 291 cases of ESBL *E. coli* infections over 12 months, 107 patients (37%) had acquired these infections before hospitalization (ICAAC, 2012). Surveillance data on Canadian outpatients showed that resistance in *E. coli* is consistently highest for antimicrobial agents that have been in use the longest time in human and veterinary medicine (Zhanel, 2000).

A retrospective analysis of *E. coli* from urine specimens collected from patients during 1997–2007 in Switzerland showed an increasing resistance trend for ciprofloxacin, trimethoprim/sulfamethoxazole, and amoxicillin/clavulanate (Blaettler et al 2009). Similarly a 30-year (1979–2009) follow-up study on *E. coli* in Sweden showed an increasing resistance trend for ampicillin, sulfonamide, trimethoprim, and gentamicin (Kronvall, 2010). Also a study done amongst US out patients showed that in 2010, *E. coli* isolates which demonstrated resistance to three, four or five antibiotics (ampicillin, cefalothin, ciprofloxacin, trimetroprim/sulfamethaxazole and amoxicillin/clavulanic acid), resistance to nitrofurantoin was observed in only 2.1%, 7.5%, and 24.1% of the isolates, respectively (Sanchez et al, 2014).

It is very likely that the antibiotic resistance problem generated during the last 60 years due to the extensive use and misuse of antibiotics is here to stay for the foreseeable fu-

ture. This view is based on theoretical arguments, mathematical modeling, experiments and clinical interventions, suggesting that even if we could reduce antibiotic use, resistant clones would remain persistent and only slowly (if at all) be out competed by their susceptible relatives(Andersson et al, 2011).

1.1 Research Question and Objectives

Urinary tract infections (UTI) commonly caused by *E coli* are a frequent reason for consulting in primary health care and remain one of the most common reasons for antibiotic prescribing(Sanchez et al, 2014). Although Belgian guidelines recommend nitrofurantoin as first-line empirical treatment for acute cystitis in women, broad-spectrum agents such as fluoroquinolones are frequently prescribed. MDR *E. coli* initially thought to be found only in hospitalized patients are now also found amongst outpatients(ICAAC, 2012). Many studies have examined the proliferation of MDR *E. coli* but few have examined the antimicrobial activity of nitrofurantoin against MDR *E. coli* isolates. Based on data from a previous study, IARG (Catry et al, 2004), the objectives of this study are to

1. Analyse the antimicrobial susceptibility testing (AST) data
2. To describe the prevalence of urinary MDR *E.coli* amongst Belgian outpatients
3. To assess the antimicrobial activity of oral antibiotics commonly used to treat UTIs against MDR urinary *E. coli* isolates
4. To assess the effect on MDR of age, gender, amount of antibiotics and number of antibiotic classes taken by the patient before urine sample collection.

2 Methodology

2.1 Data Description

The data used in this study are urinary *E. coli* isolates from the IARG study, a multi-centre cohort study that combined patient characteristics and reimbursed antibiotic therapies during the period of June 2004 to December 2005 with AST data from 16 laboratories from January to December 2005. The kirby Bauer method for AST was used by the different laboratories in the IARG study. The results of the AST were reported as: sensitive, S (if the observed zone of inhibition of *E. coli* growth was greater than or equal to the standard for that antibiotic), resistant, R (if the observed zone of inhibition of *E. coli* growth was less than the standard for that antibiotic) or intermediate, I (if the zone of inhibition of *E. coli* growth lies between that of the sensitive and resistant zone for that antibiotic).

In line with the work by Sanchez et al (Sanchez et al, 2014) in *E. coli* isolates that were tested with all the six antibiotics (nitrofurantoin (NIT), ampicillin (AMP), amoxicllin/clavulanate (AMC), ciprofloxacin (CIP), trimethoprim/sulfamethoxazole (SXT) and cefalothin (CEF)). There were a total of 16146 urinary *E. coli* isolates from the IARG study. 9654 (59.79%) of these *E.coli* isolates were tested with less than the six antibiotics of interest while 6478 (40.12%) *E. coli* isolates were tested with the six antibiotics of interest and fourteen (0.09%) of the 16146 urinary *E. coli* isolates had more than one result of AST for the same antibiotic for the same *E. coli* isolate. These fourteen isolates were left out of the analysis. Only the 6478 isolates tested with all six antibiotics of interest (NIT, AMP, AMC, CIP, SXT and CEF) were considered for the analyses.

A total of 5158 patients contributed to the 6478 *E. coli* isolates. A few 60($<$2%) of these patients had more than one *E. coli* isolate. There was some time lapse between the urine samples. Of the 6478 *E. coli* isolates that were tested with the six antibiotics of interest, 2666 (41.15%) had intermediate results to at least one of the six tested antibiotics. These

intermediate results were considered in a first analysis as non-susceptible (resistant) and later as susceptible (sensitive) for a sensitivity analysis. The response variable was defined as resistance of an *E. coli* isolate to at least three antibioctics (multidrug-resistant, MDR) or resistance to less than three antibiotic (non multidrug-resistant, non-MDR) (Sanchez et al, 2014). The covariates considered here are age and gender of the patient, the amount of antibiotic and the number of antibiotic classes (pharmacological subgroups defined by the Anatomical Therapeutic Chemical(ATC) classification amongst the antibacterial for systemic use (ATC code J01)) (WHO, 2015) taken by the patient before sample urine collection.

AGE: Age ranged from 0 to a maximum of 101 years with a mean age of about 63 years. Age was categorised into four groups as children, young adults, adults and the elderly similar to that done by Enrico et al (Enrico, 2012) as seen in Table 1

GENDER: The data consist of 1071(20.76%) males and 4087(79.23%) females.

Total Daily Defined Dose (DDD): The amount of antibiotic was defined as the total Daily Defined Dose (DDD) of all antibiotics taken by the patient before the urine sample was collected. This variable is continuous, a categorical version of the variable (DDDcat) (Boudewijn et al, 2008) was also considered as described in Table 1.

The Number of antibiotic classes (NATC): Defined as a count of the different ATC classes to which the antibiotics taken by the patient before urine sample collection belong. The ATC subgroups of the different antibiotics taken by the patients before urine sample collection are presented in Table 3.

Table 1: Variable description

Variable	Type	Description
MDR	Binary	1: resistance of *E. coli* to at least 3 antibiotics 0: resistance of *E. coli* to less than 3 antibiotic(s)
Age	Categorical	Children, 1 : 0-14years Young adult, 2 :15-29years Adults, 3 : 30-59years Elderly, 4 : >60years
Gender	Binary	1: Male 2: Female
Daily defined dose DDD	Continuous	
Daily defined dose DDDcat	Categorical	DDD0: 0 DDD1: [0.001, 9] DDD2: [9.001, 24] DDD3 [24.001, 59] DDD4 > 59
NATC	continuous	Number of antibiotic classes

2.2 Exploratory data Analysis

To get an insight into the data, plots and tables of proportions of isolates resistant to any or all of the six antibiotics were used.

2.3 McNemar's Test for proportions

McNemar's test can be used to test the difference in proportions between paired binary response data. It is applied to a 2×2 contigency table with a binary trait to check if the row and column totals are equal (marginal homogeneity) (Agretsi, 2002). Each *E. coli* isolate was tested with all the six antibiotics of interest. Therefore the proportions of isolates non-susceptible or susceptible to each of the six antibiotics are dependent. AS such the difference in these proportions can not be tested using standard procedures for independent samples thus the need for McNemar's test . To perform the McNemar's test, a 2×2 contigency table was produced as in Table 2.

Table 2: 2×2 contigency table for Mcnemar's test

	ATB_i: R	ATB_i: S	Row Total
NIT: R	a	b	a+b
NIT: S	c	d	c+d
Column Total	a+c	b+d	n

ATB_i=AMP, CEF, CIP, SXT and AMC
n=Total number of MDR *E. coli* isolates

Where, a=number of MDR *E. coli* isolates non-susceptible to both NIT and one of the other five antibiotics, b=number of MDR *E. coli* isolates non-susceptible to NIT but susceptible to one of the other five antibiotics, c=number of MDR *E. coli* isolates susceptible to NIT but non-susceptible to one of the other five antibiotics, d=number of MDR *E. coli* isolates susceptible to both NIT and one of the other five antibiotics. Assuming that the urine samples were taken from patients randomly selected from the population, and given the large sample size, five tests were performed; each comparing NIT with one of the other five antibiotics. The hypotheses tested are:

$H0 : P_i = P_{NIT}$

$Ha : P_i \neq P_{NIT}$

Where

P_i=The proportion of MDR *E. coli* isolates non-susceptible to the ith antibiotic, P_{NIT} is the proportion of MDR *E. coli* isolates non-susceptible to NIT, i= AMP, CEF, CIP, SXT and AMC.

Using the 2×2 contigency table in Table 2, the Mcnemar test statistics was calculated as in Equation 1 (Agretsi, 2002). This statistic has a χ^2 distribution with 1 degree of freedom.

$$X^2 = \frac{(b-c)^2}{b+c} \tag{1}$$

2.4 Test of Proportion in Two populations

Two population proportions can be compared using the Z test for difference in proportion, if the two proportions are from independent random samples and the sample size of the each of the two populations is sufficiently large (Walpole et al, 2013). The Z test statistic is calculated as in Equation 2

$$Z = \frac{\hat{p_1} - \hat{p_2}}{se(\hat{p_1} - \hat{p_2})} \tag{2}$$

and $se(\hat{p_1} - \hat{p_2} = \sqrt{\frac{\hat{p_1}(1-\hat{p_1})}{n1} + \frac{\hat{p_2}(1-\hat{p_2})}{n2}}$

where $se(\hat{p_1} - \hat{p_2}$ is the standard error of the proportion difference $(\hat{p_1} - \hat{p_2})$ and n_1 and n_2 are the number of observations in the two populations, respectively; $\hat{p_1}$ and $\hat{p_2}$ are the proportions with characteristic of interest in the two populations, respectively.

Assuming that patients were randomly selected from the population and that the 6478 *E. coli* isolates are independent, the proportions of MDR *E. coli* isolates between males and females; between isolates from patients below fifteen compared to those above fourteen years,

between isolates from patients with no previous antibiotic therapy and those with at least some antibiotic therapy were compared using the Z test (Z^2 has χ^2 distribution with 1 degree of freedom). The hypotheses tested are:

1. $H0 : P_{AGE1} = P_{AGE2} = \ldots P_{AGE4}$
2. $H0 : P_{males} = P_{females}$
3. $H0 : P_{DDD0} = P_{DDD1} = \ldots DDD4$
4. $H0 : P_{NATC0} = P_{NATC0} \ldots P_{NATC_7}$

Where: $P_{AGE1} \ldots P_{AGE4}$, P_{males} and P_{female} are the proportions of MDR *E. coli* isolates from patients below fifteen years, fifteen to twenty nine, thirty to fifty nine, at least sixty years, male and female patients, respectively.

P_{DDD0} ... P_{DDD4} is the proportion of MDR *E. coli* isolates from patients with no antibiotic therapy to a maximum of DDD >59, respectively before urine sample collection. $P_{NATC_0} \ldots P_{NATC_7}$ is the proportion of MDR *E. coli* isolates from patients who took none to a maximum of 7 antibiotic classes, respectively before urine sample collection.

2.5 Pearson Chi square test of association

The Pearson chi-square test of association can be used to test the hypothesis of no association between two or more groups, populations, or criteria. It compares the observed counts to the expected counts under the assumption of no association between the groups, populations, or criteria (under the assumption that the null hypothesis is true). The test statistic is calculated as in Equation 3 below.

$$X^2 = \frac{\sum_{i=1}^{r} \sum_{k=1}^{k} [O_{ik} - E_{ik}]}{E_{ik}} \tag{3}$$

Where i=number of rows, k=number of columns, O_{ik} and E_{ik} are the observed and expected cell cell counts in the ith row and the kth column, respectively. For large samples, the test statistic (X^2) has a chi-square distribution with degrees of freedom given by (r-1)(k-1). A large value of this statistic indicates that the observed data are unlikely under an assumption of no association between the two groups and a small value of the test statistic indicates no association between the two groups(Agretsi, 2002).

To test for the hypothesis of no association between age , gender, the amount of antibiotic (DDDcat), number of antibiotic classes (NATC) and MDR, a χ^2 test of association was performed. The four hypotheses tested are

1.H0: There is no association between MDR and age.

2. H0: There is no association between MDR and gender.

3. H0: There is no association between MDR and DDDcat.

4. H0: There is no association between MDR and NATC.

2.6 Correcting for multiple testing

Testing many hypotheses at the same time increases the probability of observing at least one significant result by chance (increases the probability of type I error) even if all of the hypotheses are actually not significant. The probability of observing at least one significant result by chance increases with each additional hypothesis
tested (Gelman et al, 2012).

Five comparisons were made testing the difference in the of proportions of MDR *E. coli* isolates non-susceptible to NIT and the other five antibiotics (Section 2.3), five and comparisons were made in testing the difference in the proportions of MDR *E. coli* isolates between the DDD groups, three for age groups and seven comparison for NATC groups (Section 2.4).

Therefore, to ensure that the true proportion difference were detected, the p values of the hypotheses were adjusted for multiple comparison using the Benjamin/Hochberg method. This method is less conservative with respect to Type I error but is more powerful in terms of detecting real effects. It uses the False discovery rate (FDR) to control the proportion of false positives among the set of rejected hypotheses(Gelman et al, 2012). The p values of the hypotheses are ordered from largest to smallest, and the adjusted p value for each hypothesis is calculated as in Equation 4.

$$P_{adjusted} = \frac{j}{m} * \alpha \tag{4}$$

where $P_{adjusted}$ is the adjusted p value for the jth hypothesis, j=1..m, the number of hypothesis tested and α is the overall significance level. The hypotheses with ranks 1...j are considered significant if

$$P_j \leq \frac{j}{m} * \alpha$$

where P_j is the unadjusted p value of the jth hypothesis (Tamhane et al, 1999)

For the analyses, the adjusted p values will be presented if different from the unadjusted.

2.7 Multiple Logistic Regression

The chi square test of association presented in Section 2.5, merely indicates the degree of evidence of association but does not estimate the nature and the strength of the association between the compared groups (Agretsi, 2002). In order to study the nature, estimate the strength of the association between the predictors (age ,gender, amount of antibiotics and number of antibiotics classes taken before urine sample collection) and the response variable (MDR or non MDR), to take into account multiple variables and interactions, and given that the response variable is binary a multiple logistic regression model with logit link was considered to model the outcome.

Each observation (*E. coli* isolate), y_i thus follows a binomial distribution with mean π_i, $y_i \sim Bin(\pi_i)$, where y_i is the response (1 for MDR and 0 for non-MDR *E. coli*) and π_i is the probability of a MDR *E. coli*, i=1...6478. A logistic regression model was fitted as in Model 5 below.

$$logit(\hat{\pi}_i) = \hat{\beta}_o + \hat{\beta}_1 + ...\hat{\beta_{p-1}} \tag{5}$$

where $\hat{\pi}_i$ is the estimated probability of an *E. coli* isolate to be MDR, $logit(\hat{\pi}_i)$ is the logit of the probability a MDR *E.coli* isolate, $\hat{\beta}_o$ is the intercept and $\hat{\beta}_1, ...\hat{\beta_{p-1}}$ are regression coefficients.

2.7.1 Model Building

To get valid inferences from the model, it is important to ensure that not too many variables are included in the model which may result in over fitting and lead to large

variances of parameter estimates compared to a simple model. On the other hand care has to be taken not to exclude important variables from the model which will lead to biased estimates of regression coefficients and and predictions of new observations (Kutner et al, 2005). It is therefore important to have a model which is complex enough to fit the data well and on other hand keeping it simple to interpret rather than over fitting (Agresti, 2002).

Logistic regression models were fitted and likelihood ratio tests used to choose the "best" model if the models are nested (Wagenmakers and Farrell, 2004) and Akaike information criterion (AIC)(Akaike, 1981) used if the models are not nested.

First Model1, a model with main effects only with DDD as continuous variable was fitted and compared with Model2, a model with main effects only but with DDD as a categorical variable(DDDcat). The model with a smaller AIC (the two models are not nested) between Model1 and Model2 was chosen. To assess the significance of the main effects, the chosen model between Model1 and Model2 was compared with an intercept only model using likelihood ratio test. If this test was significant this model (model1 or Model2) was compared to other models which contain main and interaction effects. Interactions effects were added one at a time to the chosen model (model1 or model2) and their significance checked using likelihood ratio tests. The hypothesis for each likelihood ratio test is as follows:

H0: Model with main effects fits well.

Ha:Model with main effects + an interaction effect fits better.

The "best" model was then checked for goodness of fit using Hosmer-Lemeshow's goodness of fit statistics, since some predictor variables are continuous (DDD and NATC) and may lead to a large number of covariate patterns resulting in cells with expected cell count less than five. Therefore Deviance and Pearson's goodness of fit statistics are unsuitable for this situation (Hosmer and Lemeshow, 2000).

2.8 Sensitivity Analysis

The response variable was defined as MDR (resistance of an *E. coli* isolate to at least three of the six tested antibiotics) or non-MDR (resistance of an *E. coli* isolate to at most three of the six tested antibiotics). However as mentioned in the section 2.1 (Data description), some *E. coli* isolates were intermediate to some antibiotics. Also only a few patients were sampled more than once and as such a full analysis of the data as clustered data was not be considered. Treating these clustered *E. coli* isolates as independent can lead to invalid (smaller) standard errors of parameter estimates and consequently invalid conclusions.

Therefore a sensitivity analysis was done to check if considering the intermediate AST results as susceptible or non-susceptible and or assuming all 6478 *E. coli* isolates as independent had an impact on the results. For the antimicrobial activity of nitrofurantoin, all 6478 observations were used but with intermediate AST results considered as susceptible. For the effect of the covariates on MDR, three logistic regressions were fitted; first assuming the 6478 *E. coli* isolates as independent and considering intermediate AST results as susceptible, secondly with 5158 observations (excluding the duplicates) and intermediate AST results as susceptible and finally with intermediate AST results as non-susceptible using independent observations (5158).

2.9 Software

R 3.0.2 and SAS 9.4 were used for the analyses. The level of statistical significance was set at 0.05.

3 Results

3.1 Exploratory data Analysis

A total number of 6478 *E.coli* isolates were considered for the analyses. Assuming these isolates as independent and the intermediates AST results as non-susceptible, there were 489 (7.55 %) *E.coli* isolates from patients below 15 years, 441 (6.81 %) from patients aged between fifteen and twenty nine years, 1184(18.28%) from patients between thirty and fifty nine years and 4364 (67.37%) isolates from patients at least sixty years. This suggests that may be most likely to isolate *E. coli* from patients at least sixty compared to those younger than sixty. Female patients accounted for a total of 5138 (79.31%) *E. coli* isolates while 1340 (20.69%) *E. coli* were isolated from male patients.
2015 (31.105%) of the *E.coli* isolates were isolated from patients who took antibiotics after urine sample collection or who had no history of antibiotics therapy before urine collection and 4463 (68.89%) isolates were from patients who had an antibiotic therapy before urine sample collection. The amount of total Daily Defined Dose range from 0 to a maximum of 641.60 with a mean of 18.5. Table 3 presents the different classes of the antibiotics taken by the patients before urine sample collection; ie pharmacological subgroups defined by the ATC classification amongst the antibacterial for systemic use (ATC code J01) . 4198 (64.80%) of the 6478 *E. coli* isolates were non-susceptible to at least one of the six antibiotics while 6420 (99.10%) of the isolates were susceptible to at least one of the six antibiotics and 2281 (35.21%) of the isolates were susceptible to all (pan- susceptible) of the six antibiotics. A total of 1946 (30.04%)) of the *E. coli* isolates were MDR while 4532 (69.96%) were non-MDR.

Table 3 shows that the different antibiotics classes taken by the patients before urine sample collection belong to 9 different ATC classes, all of which are antibiotics for systemic use (the J01 group).

Table 3: Classes* of Previous antibiotic therapy

Antibiotic class	ATC
Tetracyclines	J01A
Amphenicoles	J01B
Penicillines	J01C
Cefalosporins	J01D
Sulphonamides	J01E
Microlides	J01F
Amiglycosides	J01G
Quinolones	J01M
Nitrofuranes	J01X

*=pharmacological subgroups defined by the Anatomical Therapeutic chemical (ATC) classification amongst the antibacterial for systemic use (ATC code J01).

The number of antibiotic classes range from zero(no history of previous antibiotic therapy or antibiotic therapy on the day of urine sample collection) to a maximum 7 with a mean of 2 antibiotic classes.

3.1.1 Pattern of resistance

There were a total of 47 unique resistance patterns shown by the 6478 *E. coli* isolates. Table 4 shows the resistance patterns of pan-susceptible and MDR *E. coli* to the six antibiotic. 35(74.47%) of these patterns were unique to MDR *E. coli* isolates. As can be seen from the table, MDR *E. coli* isolates susceptible to NIT had 12 different patterns (1676 isolates) while MDR *E. coli* susceptible to AMC had 16 different of patterns(974 isolates). MDR *E. coli* were non-susceptible to AMP in almost all 37 patterns.

Table 4: Resistance Patterns of Pan-susceptible and MDR *E. coli* isolates.

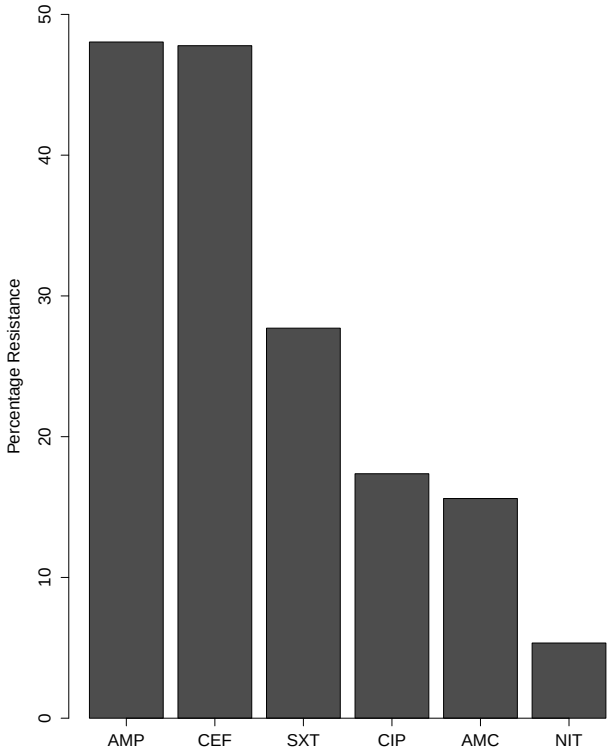
AMC	AMP	CEF	CIP	NIT	SXT	∑n	%
S	S	S	S	S	S	2281	35.21
S	R	R	S	S	R	373	5.76
R	R	R	S	S	S	306	4.72
S	R	R	R	S	R	220	3.40
R	R	R	R	S	R	216	3.33
R	R	R	S	S	R	200	3.09
S	R	R	R	S	S	125	1.93
R	R	R	R	S	S	93	1.44
S	R	S	R	S	R	72	0.01
R	R	R	R	R	R	58	0.90
S	R	R	R	R	R	45	0.70
S	R	R	S	R	R	34	0.52
S	R	R	R	R	S	26	0.40
R	R	S	S	S	R	22	0.34
R	R	S	R	S	R	21	0.32
S	R	R	S	R	S	16	0.25
S	S	R	R	S	R	16	0.25
R	R	R	R	R	S	15	0.23
R	R	R	S	R	S	15	0.23
S	R	S	R	R	R	15	0.23
R	R	S	R	S	S	12	0.19
R	R	R	S	R	R	11	0.17
S	S	R	R	R	R	8	0.12
S	R	S	R	R	S	7	0.11
S	R	S	S	R	R	7	0.11
S	S	R	R	R	S	5	0.08
S	S	R	S	R	R	3	0.05
S	S	S	R	R	R	2	0.03
R	R	S	R	R	R	1	0.02
R	R	S	R	R	S	1	0.02
R	R	S	S	R	R	1	0.02

R= resistant, S=Sensitive.
$\sum n$=Total number of *E.coli* in each pattern.
%=percentage of all isolates (n=6478).

Proportion of Resistance by antibiotic

Figure 1 shows of the overall resistance of the *E.coli* isolates to each antibiotic. We can see from the figure that a total of 346 (5.34%), 1011(15.61%), 1125(17.37%), 1795(27.71%), 3095(47.78%) and 3112 (48.04%) *E. coli* isolates were resistant to NIT, AMC, CIP, SXT, CEF and AMP, respectively.

The plot suggests that among the six antibiotics the proportion of non-susceptible *E. coli* isolates is the lowest for NIT.



NIT= nitrifurantoin, AMC=amoxicillin/clavulanate, CIP=ciprofloxacin,
SXT=trimethoprim/sulfamethoxazole, CEF=cefalothin and AMP= ampicillin

Figure 1: Bar plot of the proportion of *E. coli* non-susceptible isolates to each of the six antibiotics

Table 5: Percentage Resistance to the six Antibiotics

No. of ATB		% Resistance					
	n	NIT	AMP	AMC	CIP	SXT	CEF
1	1242	2.82	27.30	0.00	3.22	10.950	55.78
2	1010	4.06	85.25	3.86	12.57	33.07	61.19
3	966	4.14	97.31	35.20	24.74	51.24	87.37
4	634	15.77	98.74	52.208	60.57	78.71	94.01
5	288	25.00	100.00	84.38	96.18	94.79	99.65
6	58	100.00	100.00	100.00	100.00	100.00	100.00

ATB=Antibiotic, n=number of isolates resistant to 1-6 antibiotics,
n is used as the denominator in each case.

Table 5 shows the number and the proportion of non-susceptible *E. coli* isolates to one up to a maximum of six antibiotics, respectively. The table also shows the percentage of non-

susceptible *E. coli* isolates to each antibiotic in each case. The table suggests that NIT seems to have the smallest proportion of non-susceptible *E. coli* isolates in most cases. NIT seems to demonstrate consistent antimicrobial activity even against MDR isolates as it has the lowest proportion of non-susceptible isolates amongst the MDR *E. coli* isolates. It can also be seen in Table 5 that, 1242 (19.17%), 1010 (15.59%), 966(14.91%), 634(9.79%), 288(4.45) and 58(0.90%) of the 6478 *E. coli* isolates were non-susceptible to one up to a maximum of six of the antibiotics, respectively. Figure 2 shows the proportion of pan-susceptible (Pan), MDR and the proportion of *E. coli* isolates non-susceptible to one (R1) up to a maximum of six (R6) antibiotics, respectively.

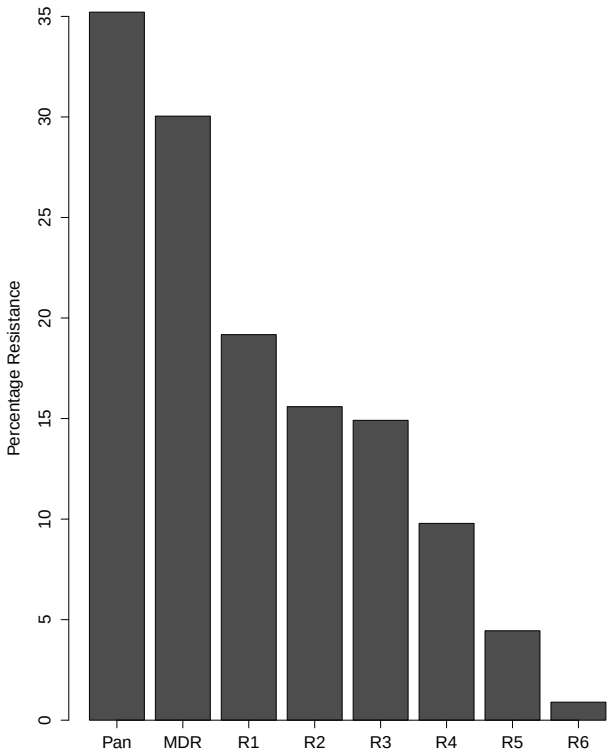


Figure 2: Bar plot of Proportion of *E. coli* Pan-susceptible (Pan) , MDR and isolates resistant to one (R1) to a maximum of six (R6) antibiotics, respectively.

Figure 2 shows that 35.21% of the *E. coli* isolates were susceptible to all the six antibiotics (pan-susceptible) while 30.04% of the isolates were MDR and very few (<1%) were non-

susceptible to all the six antibiotics.

3.2 Tests of Difference in proportions

3.2.1 Antimicrobial activity of nitrofurantoin against MDR *E. coli*

Amongst the 1946 MDR *E. coli* isolates, 270(13.87%), 1912(98.25%), 972(49.95%), 958(49.23%), 1325(68.09%) and 1785(91.73%) were non-susceptible to NIT, AMP, AMC, CIP, SXT and CEF, respectively. Figure 3 is a graphical representation of these percentages.

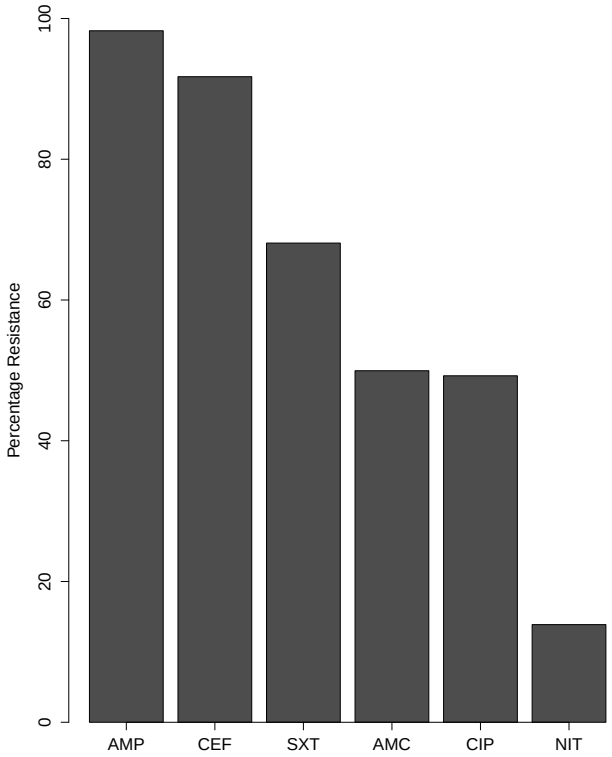


Figure 3: Bar plot of proportion non susceptibility to the six tested antibiotics amongst MDR *E. coli* isolates.

Figure 3 shows the proportion of non-susceptible isolates to each of the six antibiotics amongst the MDR *E. coli* isolates. Figure 3 suggests that amongst the six antibiotics tested, non-susceptibility to NIT is less likely.

Table 6: Difference in proportion isolates non-susceptible to NIT and the other five antibiotics amongst MDR *E. coli* isolates

Antimicrobial	LCL	DIFF	UCL	Unadjusted p.value
NIT Vs AMP	-0.86	-0.84	-0.83	<0.0001
NIT Vs AMC	-0.39	-0.36	-0.33	<0.0001
NIT Vs CIP	-0.38	-0.35	-0.32	<0.0001
NIT Vs SXT	-0.57	-0.54	-0.52	<0.0001
NIT Vs CEF	-0.80	-0.78	-0.76	<0.0001

LCL=Lower confidence interval
UCL=Upper confidence interval
DIFF=difference in proportion)

Table 6 presents the difference in the proportion between MDR *E. coli* isolates non-susceptible to NIT and the other five antibiotics tested antibiotics, 95% confidence intervals of these differences and the p values for the Mcnemar test of difference in proportion. The table shows that NIT has the least number of non-susceptible isolates amongst the MDR *E. coli* isolates as compared to the other five antibiotics as can be seen from the negative differences in proportions, confidence intervals which do not include zero and also the unadjusted p values (the adjusted p values were same as the unadjusted) which are all <0.05.

This shows that the antimicrobial activity of NIT is preserved even against MDR E. coli isolates as was also suggested in Table 5 and Figure 3.

3.2.2 Difference in proportion of MDR *E. coli* by gender, age group, amount of antibiotic and by number of antibiotic classes

The difference in the proportion of MDR *E. coli* by gender, age group, DDD group and number of antibiotics classes, the 95% confidence interval of the differences and the p values for the chi square (Z^2) tests for these differences are presented in Table 7. Table 7 shows that 461 (34.34%) of the *E. coli* isolates from male patients are MDR while 1485 (28.90%) of the *E. coli* isolated from females are MDR. The table also suggests that the proportion of MDR *E. coli* from the age groups below fifty nine are not different from

each other. It can be seen from the table that the number of MDR *E. coli* seems to increase with increase in the amount antibiotic and number of antibiotic classes.

Table 7: Proportion difference in MDR isolates by gender, age group, DDD group and number of antibiotics classes before urine sample collection

	MDR	n	LCL	DIFF	UCL	P value
GENDER						
Male	461(34.40%)	1340				
Female	1485(28.90%)	5138	0.03	0.06	0.08	<0.0001
AGE						
0-14	119(24.34%)	489				
15-29	92(20.86%)	441	-0.02	0.03	0.09	0.21
30-59	296(25.00%)	1184	-0.051	0.0006	0.04	0.77
>60	1439(32.97%)	4364	-0.126	-0.09	-0.05	0.0001
Daily Defined Dose						
DDD0	434(20.84%)	2083				
DDD1	399(28.98%)	1377	-0.11	-0.08	-0.05	<0.0001
DDD2	419(32.61%)	1285	-0.15	-0.12	-0.09	<0.0001
DDD3	445(36.15%)	1219	-0.19	-0.16	-0.12	<0.0001
DDD4	249(48.44%)	514	-0.32	-0.28	-0.23	<0.0001
Number of ATB classes						
0	434(20.84%)	2083				
1	454(27.27%)	1665	-0.09	-0.07	-0.04	<0.0001
2	418(32.97%)	1268	-0.15	-0.12	-0.09	<0.0001
3	306(39.59%)	773	-0.23	-0.19	-0.15	<0.0001
4	179(46.86%)	382	-0.31	-0.26	-0.21	<0.0001
5	112(48.28%)	232	-0.34	-0.27	-0.34	<0.0001
6&7	43(57.33)	75	-0.48	-0.37	-0.25	<0.0001

LCL=Lower confidence interval.
UCL=Upper confidence interval.
DIFF=difference in proportion. ATB=antibiotic
n=Total number of *E. coli* isolates in each group.

Table 7 shows that there is a statistically significant (all p values <0.05) and possibly clinically relevant difference in proportion of MDR isolates between males and females, the DDD groups and the antibiotic classes. There were significantly (p value <0.05) more MDR *E. coli* isolated from patients at least 60 years compared to the other age groups below sixty.

3.3 Bivariate Analysis

Table 8: Chi-square of association between gender, age, amount of antibiotic, number of antibiotics classes and MDR

	MDR	NONMDR	χ^2 statistic	Unadjusted P value
Gender				
Male	461(7.12%)	879(13.57%)	-	-
Female	1485(22.92%)	3653(56.39%)	15.04	0.0001
AGE				
0-14	119(1.8%)	370(5.71%)	-	-
15-29	92(1.42%)	349(5.39%)	-	-
30-59	296(4.57%)	888(13.71%)	-	-
>60	1439(22.21%)	2925(45.15%)	57.44	<0.0001
Daily Defined dose				
DDD0	434(6.70%)	1649(25.46%)	-	-
DDD1	399(6.16%)	978(15.09%)	-	-
DDD2	419(6.24%)	866(13.37%)	-	-
DDD3	445(6.87%)	774 (11.95%)	-	-
DDD4	249(3.84%)	265(4.09%)	195.83	<0.0001
No. of ATB classes				
0	434(6.70%)	1649(25.46)	-	-
1	454(7.01)	1211(18.69)	-	-
2	418(6.45)	850(13.12)	-	-
3	306(7.21)	467(4.72)	-	-
4	179(2.76)	203(3.13)	-	-
5	112(1.73)	120(1.85)	-	-
6&7	43(0.66)	32(0.49)	243.46	<0.0001

LCL=Lower confidence interval.
UCL=Upper confidence interval.
ATB= antibiotic

Table 8 presents the number(percentage) of MDR and non MDR *E. coli* isolates by gender, age, amount of antibiotic and number of antibiotics classes taken before urine sample collection. The table also shows the test statistic, the p values of the χ^2 test of association between age, gender, amount of antibiotics, number of antibiotic classes taken before urine sample collection. It can be seen from the table that the number of MDR *E. coli* isolates were similar across the five Daily Defined Dose groups (see Table 1 for variable description) except for the last category which has 3.84% (n=6478) of the

MDR *E. coli* isolates as compared to about 6% for the other four categories. Table 8 also shows that there is a statistically significant association between gender, age, amount of antibiotic, number of antibiotic classes and MDR as can be seen from the unadjusted p values which are all <0.05 (adjusted p values were same as the unadjusted p values).

3.4 Statistical Analysis

3.4.1 Model Building

Model1 had a smaller AIC (7646.13) value compared to Model2 (7656.48), Model1 was thus chosen as a better model over Model2. Comparing Model1 with an intercept only model showed that at least one of the main effects had a significant effect on the response (p value for the likelihood ratio test <0.0001) and Wald tests showed that all main effects were significant(p values < 0.05). Model1 was then compared with other 7 logistic regression models as can be seen in Table 9.

Table 9: Likelihood ratio test statistics and AIC values for model building

Model	Paramters	DF	-2ℓ	G^2	P value	AIC
1	Age, Gender, DDD, NATC	-	7632.13	-	-	7646.13
3	Age, Gender, DDD, NATC, DDD*NATC	1	7629.51	2.62	0.11	7645.51
4	Age, Gender, DDD, NATC ,Age*NATC	3	7631.97	0.16	0.98	7651.97
5	Age, Gender, DDD, NATC ,Age*DDD	3	7630.71	1.40	0.70	7650.71
6	Age, Gender, DDD, NATC ,Gender*DDD	3	7630.42	1.90	0.59	7646.42
7	Age, Gender, DDD, NATC ,Gender*NATC	3	7631.75	0.54	0.91	7647.75
8	Age, Gender, DDD, NATC, Age*Gender	3	7626.46	5.66	0.13	7646.46

Table 9 shows the the fitted model, the parameters in each model, the degrees of freedom (DF) of the likelihood ratio test , the -2 log-likelihood (-2ℓ) of each model, the difference in -2 loglikelihood (Deviance, G^2) between Model1 and each of the other fitted models and the AIC values for each of the 7 fitted logistic regressions models. As can be seen in Table 9, likelihood ratio tests comparing Model1 to the other 7 models all have p values greater than 0.05. Therefore none of the 7 models compared was better than Model1.

Model1 was thus selected as the "best model" and is represented in Equation 6 below

$$logit(\hat{\pi}_i) = \hat{\beta}_0 + \hat{\beta}_1 * age + \hat{\beta}_2 * gender + \hat{\beta}_3 * DDD + \hat{\beta}_4 * NATC \quad (6)$$

Where $\hat{\pi}_i$ is the estimated probability for the ith *E.coli* isolate to be MDR, i=1...6478. $\hat{\beta}_0$ is the intercept, $\hat{\beta}_1$, $\hat{\beta}_2$, $\hat{\beta}_3$ and $\hat{\beta}_4$ are the effects of the patient's age, gender, the amount of antibiotic and the number of antibiotic classes, respectively on the probability of a MDR *E.coli* isolate. The Hosmer-Lemeshow's goodness of fit statistics showed that the Model1 was of good fit (p value 0.54 >0.05).

3.4.2 Multiple Logistic Regression

Table 10: Parameter estimates, standard errors and p values of the fitted model assuming all 6478 *E. coli* as independent

	Intermediate AST results as non-susceptible			Intermediate AST results as susceptible		
Parameter	Estimate	Standard	P value	Estimate	Standard	P value
Intercept	-1.35	0.06	<.0001	-2.29	0.07	<.0001
Age2 vs 1	-0.18	0.10	0.05	-0.28	0.14	0.04
Age3 vs 1	-0.06	0.07	0.33	-0.10	0.09	0.27
Age4 vs 1	0.24	0.05	<.0001	0.46	0.07	<.0001
Gender	-0.09	0.03	0.01	-0.10	0.040	0.02
DDD	0.004	0.001	0.0002	0.01	0.001	<.0001
NATC	0.20	0.02	<.0001	0.21	0.03	<.0001

Table 10 presents the parameter estimates, standard errors and p values of the fitted logistic regression, Model1. The table shows assuming all 6478 *E. coli* to be independent and intermediate AST results as non-susceptible, age, gender, the number of antibiotic classes (NATC) and the amount of antibiotic (DDD) taken by the patient before urine sample collection, have a significant effect on the probability of a MDR *E. coli* isolate.

Table 11: Odds ratios of the fitted Logistic Regression

Effect	Point Estimate	95% LCL	95% UCL
Age 2 vs 1	0.83	0.61	1.14
Age 3 vs 1	0.93	0.73	1.20
Age4 vs 1	1.27	1.02	1.58
Female vs male	0.83	0.73	0.95
DDD	1.00	1.00	1.01
NATC	1.23	1.17	1.29

LCL=Lower confidence Limit
UCL=Lower confidence Limit

Table 11 presents the odds ratio estimates of the fitted model and their 95% confidence interval. We can see from the table that there is no significant difference between the odds of MDR *E. coli* for patients between fifteen and twenty nine years, patients between thirty and fifty nine, respectively compared to patients below fifteen years, as can be seen from their 95% confidence intervals which include one. However patients at least sixty have a significantly higher odds (27%) of MDR compared to patients below fifteen years, as the 95% confidence interval of this odds ratio does not include one. Table 11 also shows that it is less likely to isolate a MDR *E. coli* from female patients compared to male patients. The table shows that the risk of MDR *E. coli* does not increase for a unit increase in DDD but for a 10 fold increase in the amount of antibiotic (DDD) taken before urine sample collection, the odds of a MDR *E. coli* increases by 4.08% meaning the risk of MDR increases with a ten fold increase in the amount of antibiotic (DDD) taken before urine sample collection. For a unit increase in the number of antibiotic classes the odds of a MDR *E. coli* increases by 23%, therefore risk of MDR *E. coli* increases with increase in the number of antibiotic classes taken before urine sample collection.

3.5 Sensitivity analysis

When the intermediate AST results were considered as susceptible, 3499 (54.01%) of the 6478 *E. coli* isolates were resistant to at least one of the six antibiotics tested while

6459(99.71%) were susceptible to at least one of the six tested antibiotics. 2980 (46.00%) of the 6478 *E. coli* isolates were pan-susceptible. Table 12 shows the number of non-susceptible *E. coli* isolates to one up to a maximum of six tested antibiotics .

Table 12: Percentage of resistance to the six antibiotics tested considering intermediate AST results as susceptible

No. ATB	n	% Resistance					
		NIT	AMP	AMC	CIP	SXT	CEF
1	1217	1.97	71.24	0	7.97	14.30	4.52
2	1163	1.46	94.15	2.92	17.63	63.80	20.03
3	691	4.63	99.28	17.22	61.65	70.91	46.31
4	281	15.30	100	33.10	82.56	86.83	82.20
5	128	24.22	100	78.91	99.22	98.44	99.22
6	19	100	100	100	100	100	100

ATB= Antibiotic
n=number of isolates resistant to one up to a maximum
of six antibiotics and n is used as the denominator in each case.

Table 12 shows that 1119(17.27%) of the 6478 *E. coli* isolates were MDR while 5359(82.73%) of the isolates were non MDR. It can be seen from 12 that non-susceptibility to NIT seems to be the least as was seen earlier in Table 5 when intermediate AST results were considered as non-susceptible.

This is confirmed by the test of difference in proportion in Table 13 which shows that non-susceptibility against NIT amongst MDR *E. coli* isolates is significantly lower as compared to the other five antibiotics.

3.5.1 Diffference in proportion of MDR *E. coli* isolates

Table 13: Antimicrobial Activity of NIT compared to the other five antibiotics considering intermediate AST results as susceptible

	LCL	DIFF	UCL	unadjusted p value
NIT Vs AMP	-0.84	-0.82	-0.79	<0.0001
NIT Vs AMC	-0.44	-0.40	-0.36	<0.0001
NIT Vs CIP	-0.57	-0.54	-0.51	<0.0001
NIT Vs SXT	-0.54	-0.61	-0.57	<0.0001
NIT Vs CEF	-0.73	-0.70	-0.67	<0.0001

LCL=Lower confidence interval
UCL=Upper confidence interval
DIFF=difference in proportion

3.5.2 Multiple logistic regression

Table10 also presents the parameter estimates, standard errors and p values of the fitted logistic regression Model1 with all 6478 *E. coli* assumed to be independent and intermediate AST results considered as susceptible . The Hosmer-Lemeshow's goodness of fit test showed that fitted Model1 with data for sensitivity analysis is also of good fit (p value = 0.85). The table shows that considering the intermediate AST results as susceptible leads to the same conclusions as when the intermediate AST results are considered as non-susceptible.

Table 14: Parameter estimates, standard errors and p values of logistic regression (Model1) using independent observations (5168)

	Intermediate result as non-susceptible			Intermediate result as susceptible		
Parameter	Estimate	Standard	P value	Estimate	Standard	P value
Intercept	-1.40	0.06	<.0001	-2.38	0.08	<.0001
Age2 vs 1	-0.19	0.10	0.06	-0.24	0.14	0.089
Age3 vs 1	-0.11	0.07	0.11	-0.15	0.10	0.109
Age4 vs 1	0.20	0.05	0.0002	0.41	0.07	<.0001
Gender	-0.07	0.04	0.08	-0.09	0.05	0.05
DDD	0.005	0.001	0.0007	0.005	0.001	0.0002
NATC	0.28	0.03	<.0001	0.30	0.03	<.0001

Table 14 shows the parameter estimates standard errors and p values of the logistic regression (Model1) fitted with independent observations. Hosmer-Lemeshow's goodness of fit statistics showed that the fitted models were of good fit (p value >0.05). The table shows that considering the intermediate AST results susceptible or non-susceptible, the effect of gender on MDR is insignificant (p value >0.05) while considering the intermediate AST results as as susceptible the effect of gender is borderline (p value $=0.05$). The direction of the effect however is the same in all cases (it is less likely to isolate MDR *E. coli* from females compared to males). All other conclusions are the same as when all 6478 *E. coli* isolates are considered as independent.

4 Discussion and Conclusion

This study used data from a previous study(IARG), 6478 *E. coli* isolates were considered for the analysis. If the intermediate results for AST are considered as non-susceptible, 30.04% (1946) of the isolates were MDR while 69.96% (4532) were non-MDR. This is line with what was reported at the Inter science Conference on Antimicrobial agents and Chemotherapy (ICAAC) in 2012 that MDR *E. coli* initially thought to be found only in hospitalized patients are now also found amongst outpatients. Also in 2012 it was shown that the prevalence of MDR *E. coli* isolates in Belgian patients was 17% of the 126 isolates considered and that MDR *E. coli* strains had spread in the entire Euregion (Christina, 2012).

Amongst the isolates that showed resistance to three, four or five antibiotics, only 4.14%, 15.77% and 25% were resistant to nitrofurantoin, while wide-spread resistance was seen with ampicillin (97.31%, 98.74% and 100%), amoxicillin/clavulanate (35.20%, 52.21%, 84.38%), ciprofloxacin (24.74%, 60.57% , 96.18%) , trimethoprim/sulfamethazole (51.24%, 78.71%, 94.79%) and cefalothin (87.37%, 94.01% and 99.65%) for isolates resistant to three, four or five antibiotics, respectively.

The analysis showed that the proportion of *E. coli* non-susceptibility to nitrofurantoin amongst the MDR *E. coli* isolates is significantly lower compared to the other five antibiotics. These results are similar to what was found by Sanchez and others in 2014 in a similar study in US outpatients.

Wide-spread resistance to ampicillin, amoxicillin/clavulanate, ciprofloxacin, trimethoprim/sulfamethazole and cefalothin could be an indication that resistance to an antibiotic increases with the length of time the antibiotic has been used. This was the same conclusion arrived at by Zhanel in 2000 working on surveillance data on Canadian outpatients. He showed that resistance in *E. coli* is consistently highest for antimicrobial agents that have been in use the longest time in human and veterinary medicine. This wide-spread resistance to amoxicillin/clavulanate, ciprofloxacin,

trimethoprim/sulfamethazole and ampicllin was also noticed by Blaetter et al in 2009 in a retrospective analysis of urine specimens collected from 1997-2007 in Switzerland; and Kronval in 2010 in a thirty- year (1979-2009) follow up study on *E. coli* in Sweden. In 2012 it was also shown that amongst Belgian patients resistance of urinary *E. coli* to amoxicillin-clavulanic acid was 39% and 40% to fluoroquinolones while resistance to nitrofurantoin was less than 5%, this study also indicated that most antibiotics used as first choice oral empiric treatment for UTIs (amoxicillin/clavulanate, fluoroquinolones and folate antagonists) are not appropriate for this purpose (Christiana, 2012).

Our analysis also showed that the risk of MDR *E. coli* increases with a unit increase in the number of antibiotic classes and with a ten fold increase in the Daily defined Dose (DDD) taken by the patient before urine sample collection. This is expected as was shown by Levy et al in 2004 and Cassier et al in 2011 that an over consumption of broad-spectrum antibiotics such as fluoroquinolones and certain cephalosporins can lead to increase in resistance to these antibiotics. It was also seen that males it is more likely to isolate an MDR *E. coli* isolate from males compared to females.

Patients at least sixty year old have a higher risk of MDR compared to patients below sixty, but there is no significant difference in the risk of MDR *E.coli* in patients below fifteen compared to those between fifteen and fifty nine years.

Sensitivity analysis showed that the antimicrobial activity of nitrofurantoin is preserved even when the intermediate AST results are considered as susceptible. It was also shown that considering the intermediate AST results as susceptible did not change the conclusions regarding the effects of age, gender, amount of antibiotic and number of antibiotic classes on the risk of MDR *E. coli*. It was further shown that considering only independent *E. coli* isolates had only a slight impact on the analysis (gender became insignificant). This slight effect may be due to the fact that only few of the patients had clustered *E. coli*.

In conclusion, the analysis showed that nitrofurantoin demonstrates consistent antimicrobial activity against MDR E. coli but wide-spread resistance was seen with amoxicillin, ciprofloxacin, trimethoprim/sulfamethoxazole, ampicllin and cefalothin regardless of weather intermediate AST results are considered as susceptible or non-susceptible. Thus nitrofurantoin is very reliable in treating uncomplicated cystitis compared to the other five antibiotics considered. The risk of MDR *E. coli* increases with in the number of antibiotic classes taken by the patient before urine sample collection, the risk of MDR *E. coli* also increases with a ten fold increase in the amount of Daily defined Dose. Patients at least sixty years have a significantly higher risk of MDR *E. coli* compared to those younger than sixty. Male patients have a significantly higher risk of MDR *E. coli* than female patients.

Considering intermediate results as susceptible and did not have an impact on the conclusions of the analysis but or excluding clustered observations had a slight impact on the results.

5 Limitations and Further Research

Not all data on antibiotic therapy was available as only antibiotics reimbursed were considered, as such information on amount of antibiotic and number of antibiotic classes taken by the patient may not be complete. It may also be important to consider the length of time of antibiotic consumption in order to accurately evaluate the effect of amount of antibiotic consumed on MDR *E. coli*.

Also the total Daily Defined Dose was used to represent the amount of antibiotic taken by the patient before urine sample collection. DDD may not be the best indicator for antibiotic consumption as it may not always correspond to the prescribed dose. Prescribed Daily Dose (PDD) may be a better indicator of antibiotic consumption.

Some few patients had clustered observations, had all patients been sampled more than once, random effects model will be an appropriate way to analyse the data in in order to account for account for this clustering.

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