Original Study

Prevalence and Spread of Multidrug Resistant Escherichia coli Isolates Among Nursing Home Residents in the Southern Part of the Netherlands

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\textbf{A B S T R A C T}

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Objectives: Empiric antibiotic treatment should be based on recent surveillance data. Therefore, we conducted a surveillance of (multidrug) resistance of \textit{Escherichia coli} and antibiotic use among Dutch nursing home (NH) residents. Pulsed-field gel electrophoresis and multilocus sequence typing were used to describe the spread of multidrug-resistant strains.

Design: Observational study.

Setting: Five NHs in the southern part of the Netherlands.

Participants: A total of 337 NH residents from both somatic and psychogeriatric wards.

Measurements: The prevalence and spread of antibiotic resistance and multidrug resistant \textit{E. coli} isolates collected from urine samples and antibiotic use among the NH residents were investigated.

Results: A total of 208 \textit{E. coli} isolates were collected from 308 urine samples. Resistance to amoxicillin-clavulanic acid was 23% and resistance to ciprofloxacin was 16%. Resistance totrimethoprim-sulfamethoxazole was 19%, whereas nitrofurantoin resistance was less than 1%. Multidrug resistance was observed in 28 of the 208 isolates (13%). Several isolates showed a similar pulsed-field gel electrophoresis pulsotype and multilocus sequence typing type. Sequence type (ST) 131 was the most prevalent (48%) and was demonstrated in all NHs and with four different pulsotypes. Consumption of antibiotics for systemic use was 64.4 defined daily dose (DDD)/1000 residents/day. Amoxicillin-clavulanic acid was most frequently prescribed (20.92 DDD/1000 residents/day), followed by the quinolones (14.8 DDD/1000 residents/day).

Conclusion: We observed a high prevalence of antibiotic resistance and antibiotic use. In particular, the use of resistance to fluoroquinolones is concerning. Because of the high prevalence of resistance, many agents are no longer suitable for empiric treatment. \textit{E. coli} ST131, which has also been demonstrated in this study, poses a potential risk to this vulnerable population. We have clearly demonstrated that the resistance among NH residents is different from elderly living at home and hospitalized patients, and with the emergence of resistant strains, such as ST131, NHs are a potential reservoir for multidrug resistant bacteria.

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hospitals. This might result in a relatively high use of inappropriately prescribed broad-spectrum antibiotics, of which especially the use of fluoroquinolones is a point of concern.\(^4\) These agents are often used because of their favorable pharmacokinetics and antibacterial spectrum, but resistance can be rapidly acquired, limiting the use of these antimicrobials.\(^5\) Overall, the high use of antibiotics contributes to an increase of antimicrobial resistance and a more prevalent carriage of extended spectrum beta-lactamases (ESBLs),\(^7,8\) such as the multidrug resistant ESBL producing Escherichia coli sequence type (ST) 131.\(^9,10\)

Although antibiotic use is considered as one of the main risk factors for emergence of and colonization with antibiotic-resistant isolates,\(^7\) other risk factors including poor functional status, presence of wounds, or foreign materials are also very prevalent among NH residents.\(^5,11,12\) Infections with (multidrug) resistant pathogens have been associated with higher morbidity, mortality, and costs because of delayed adequate antibiotic treatment.\(^3,14\)

UTIs are often caused by the resident’s own commensal microorganisms.\(^15,16\) Therefore, actual data of resistance of the commensal flora will guide physicians in making an appropriate choice for empiric therapy. Because current resistance data for E. coli, a commensal and the most prevalent causative agent of UTIs,\(^17,18\) are not available for NH residents in the Netherlands, we performed a surveillance in five NHs in the southern part of the Netherlands. Moreover, spread of multidrug resistant isolates, such as ST131, between and within NHs was investigated with multilocus sequence typing and pulsed-field gel electrophoresis.

**Methods**

Five NHs in the province of Limburg in the southern part of the Netherlands agreed to participate in this study. All psychogeriatric and somatic residents of these NHs were eligible for participation, but only those residents who signed a consent form (either by themselves or their legal representative) were included in the study. We received a total of 336 consent forms (Table 1). Because of withdrawal of consent, fecal incontinence, moving or death of a resident, 29 residents could not be sampled. In each NH, the samples were collected in 1 week in the period between February 2010 and June 2011. This study was approved by the medical ethics committee of the Maastricht University Medical Centre (reference no. 07-4-012.4/pl).

**Bacterial Isolates**

Urine samples were collected from all included, asymptomatic residents and used to inoculate a urict (Biomerieux, Marcy l’Etoile, France). A urict or dipslide is a semiquantitative microbial culture method, which has a two-sided paddle in a protective vial with on one side a cystine lactose electrolyte-deficient agar and on the other side a MacConkey agar.

If a resident suffered from urine incontinence, the urict was inoculated by pressing the paddle onto the incontinence pads. The inoculated uricuits were sent to the laboratory at the Maastricht University Medical Centre and incubated at 35°C for 18 hours. The putative E. coli colonies were identified using standard microbiological methods. Samples were analyzed anonymously; therefore, clinical data were not available.

**Quantitative Susceptibility Testing**

Antimicrobial susceptibility testing was performed using a microbroth dilution with Mueller-Hinton II cation-adjusted broth (Becton-Dickinson, Sparks, Maryland) and microtiter plates with freeze-dried antibiotics (MCS Diagnostics BV, Swalmen, the Netherlands). E. coli ATCC (American Type Culture Collection) 35218 and ATCC 25922 were used as control strains. The following antimicrobial agents were tested: amoxicillin, amoxicillin-clavulanic acid, ciprofloxacin, gentamicin, nitrofurantoin, norfloxacin, trimethoprim, and trimethoprim-sulfamethoxazole. The minimal inhibitory concentration data were analyzed using clinical breakpoints defined by the European Committee on Antimicrobial Susceptibility Testing (EUCAST).\(^19\) Amoxicillin-clavulanic acid–resistant isolates were further tested for resistance to ceftazidime, and ESBL production was confirmed for isolates with a ceftazidime minimal inhibitory concentration >2 mg/L using a combination disk diffusion test according to guidelines of the Dutch society for medical microbiology.\(^20\) Multidrug resistance was defined as resistance to three or more of the tested antimicrobial classes.

**Molecular Characterization**

The ESBL-positive isolates were further analyzed for the presence of bla\(_\text{TEM}\), \(\text{bla}_{\text{SHV}}\), and \(\text{bla}_{\text{CTX-M}}\) with PCR and specific primers.\(^21–24\) Automated sequencing was performed with the 3730 DNA analyzer with BigDye Terminotor v1.1 (Applied Biosystems, Foster City, California). All multidrug-resistant isolates and ESBL producers were analyzed with pulsed-field gel electrophoresis\(^25\) and multilocus sequence typing.\(^26,27\)

**Use of Antimicrobial Agents**

Data on the use of antibiotics were collected from each NH from the period of 1 year before the collection of the urine samples. These data were collected anonymously and could not be linked to an individual resident. These data were collected using the anatomical therapeutic chemical classification system/defined daily dose (ATC/DDD) classification protocol as defined by the World Health Organization Collaborating Centre for Drug Statistics Methodology.\(^28\) The DDD is the assumed average dose per day for a drug used for its main indication in adults. In this study, the DDDs are expressed as DDD per 1000 residents per day. The DDD is a unit of measurement and does not necessarily reflect the recommended or prescribed daily dose.\(^28\)

**Statistical Analysis**

To determine significant differences in prevalence of resistance, a Pearson \(\chi^2\) or Fisher exact test was performed (SPSS software 18.0; IBM, Armonk, New York). A \(P\) value <0.05 was considered statistically significant.

**Results**

In total, 208 E. coli isolates were collected ranging from 20 to 64 isolates per NH from 308 urine samples from residents of the 5 NHs (Table 1).

The prevalence of resistance is shown in Figure 1. Amoxicillin-clavulanic acid resistance varied from 14% to 31% (\(P = 0.023\)).
Ciprofloxacin ranged from 9% to 24% \((P = 0.056)\), and resistance to norfloxacin ranged from 11% to 28% \((P = 0.042)\). Resistance to trimethoprim ranged from 6% to 35% \((P = 0.010)\) and from 6% to 30% \((P = 0.008)\) for trimethoprim-sulfamethoxazole. Nitrofurantoin resistance was less than 1%. Overall resistance was greatest among residents from NH2. ESBL production was observed in 1 isolate from NH4 (<1%), which was ST38 and CTX-M 15 positive.

Multidrug resistance was observed in 28 of the 208 isolates (13%) and ranged from 9% in NH4 to 22% in NH2. All of these isolates were resistant to norfloxacin and amoxicillin. Sixty-eight percent was resistant to ciprofloxacin, 43% to amoxicillin-clavulanic acid, and 86% to the folate antagonists (Table 2). Among these isolates, ST131 was the most prevalent ST (n = 14; 50%) and was observed in each NH but with three different pulsotypes, each of which was present in one or three NHs. ST69 (n = 5) was demonstrated in three NHs. The other STs had only one pulsotype and were demonstrated in one NH only (Fig. 2).

The average consumption of antibiotics for systemic use in the 5 NHs was 64.4 DDD/1000 residents/day ranging from 52.81 to 81.56 DDD/1000 residents/day (Table 3). Antibiotics in class J01C were most frequently prescribed (20.92 DDD/1000 residents/day), followed by the quinolones (14.8 DDD/1000 residents/day) and tetracyclines (11.13 DDD/1000 residents/day). Antibiotic use of antimicrobials in class J01C ranged from 21.6 to 41.4 DDD/1000 residents/day; 81% of antibiotic use in this class could be attributed to the use of amoxicillin-clavulanic acid. Antibiotic use of antimicrobials in class J01E ranged from 1.4 to 5.9 DDD/1000 residents/day. The majority (ie, 71%) could be attributed to the use of trimethoprim, which is solely prescribed for the treatment of UTI. Use of antimicrobials in class J01M, mainly ciprofloxacin and norfloxacin, ranged from 9.8 to 23.4 DDD/1000 residents/day. Antibiotic use of antimicrobials in class J01X ranged from 1.6 to 12.2 DDD/1000 residents/day, of which 98% was nitrofurantoin.

### Discussion

This study provides the current situation of antibiotic resistance and spread of multidrug resistant *E. coli* isolates in NHs in the southern part of the Netherlands. Resistance to the folate antagonists, amoxicillin-clavulanic acid, and fluoroquinolones was mostly more than 20%, and these agents are, therefore, no longer suitable for empiric treatment of UTI.29 The only exception is nitrofurantoin because resistance was only sporadically observed. Multidrug resistance was demonstrated among 13% of the isolates, of which ST131 was the most prevalent type. This ST was observed in all NHs. The antibiotics most often prescribed were penicillins in combination with a beta-lactamase inhibitor and fluoroquinolones, which is in line with the high prevalence of resistance to these agents.

Bias of the results between the NHs because of differences in methodology was prevented by applying the same method of sampling and analysis of the urine samples for all NHs. Unfortunately, this study was performed anonymously; therefore, detailed clinical data were not available, and linkage of the antibiotic use to an individual resident was not possible.

An up-to-date overview of antibiotic resistance is necessary for a suitable empiric antibiotic treatment policy. However, if such an overview is not available, other inappropriate data are used. Resistance data from (elderly) general practice (GP) patients were different from the data presented in this study and are, therefore, not suitable as basis for empiric antibiotic choice for NH residents. The prevalence of resistance to amoxicillin, amoxicillin-clavulanic acid, and the quinolones was lower among elderly GP patients.30 This suggests that age alone is not a risk factor for antibiotic resistance, but that other factors contribute to increase of resistance. Therefore, the difference in resistance between NH residents and GP patients might be because of the high use of antibiotics among the NH residents—in particular, the high use of quinolones among NH residents compared with the GP patients,31 which is due to this agent’s oral availability, favorable pharmacokinetics, and the broad-spectrum antibacterial activity. In contrast, quinolone resistance was greater among the NH residents.
residents in all participating NHs, as was ST405, another previously especially ST131,9 is the high prevalence of resistance to the often infections such as UTIs and bacteremia.9,10,35 The same applies for options is limited. Globally, the to treat because the number of adequate antimicrobial treatment producing strains. Infections with these bacteria are more dif spread of multidrug resistant, ESBL-, and/or carbapenemase- ATC, anatomical therapeutic chemical classification system; NH, nursing home.

Compared with hospitalized patients,31 whereas antibiotic use was higher among the hospitalized patients.31 This suggests that other factors are also playing a role in the observed differences in resistance between the elderly CP patients and hospitalized patients and the NH residents. Factors such as a chronic, poorer physical status of the NH residents are also of importance. In turn, this could also explain the differences in antibiotic resistance between the participating NHs that cannot be attributed to differences in antibiotic use. Overall antibiotic use among the participating NH residents in this study could be ranked average or high compared with NH residents in other European countries.32

With regard to initiation of empiric treatment with a specific antimicrobial agent, resistance should not exceed 20% for an uncomplicated UTI and should not exceed 10% for a complicated UTI.29 Based on the resistance data, nitrofurantoin could be an appropriate agent for both uncomplicated and complicated UTI. However, because the tissue penetration of nitrofurantoin is very low,33 this agent is not suitable for the treatment of complicated UTI. Taking into account the 20% and 10% cutoff values, we found that amoxicillin-clavulanic acid, ciprofloxacin, norfloxacin, trimethoprim, and trimethoprim-sulfamethoxazole are not appropriate anymore for treatment of complicated UTI, but some agents might be appropriate for empiric treatment of uncomplicated UTI. This was, however, NH dependent. An alternative option for oral empiric treatment includes fosfomycin, which, up until now, is not registered for the treatment of complicated UTI despite promising characteristics, that is, low resistance,30 high tissue penetration, and activity against ESBL-producing isolates.34

Another point of concern is the global increase of resistance and spread of multidrug resistant, ESBL-, and/or carbapenemase-producing strains. Infections with these bacteria are more difficult to treat because the number of adequate antimicrobial treatment options is limited. Globally, the E. coli ST131 strains are often multidrug resistant, produce ESBLs, have many virulence traits, and cause infections such as UTIs and bacteremia.9,10,35 The same applies for ST69.35,36 Both ST131 and ST69 strains were found among the NH residents in all participating NHs, as was ST405, another previously reported resistant ST.36 A concerning property of these strains, especially ST131,9 is the high prevalence of resistance to the often prescribed fluoroquinolones, which was also demonstrated in this study. Fortunately, the resistant isolates observed in our population were not ESBL producers, and they were also resistant to fewer antibiotics than other previously reported multidrug resistant strains.9 We did demonstrate, however, that especially ST131 has spread into the NH population. This ST can also easily pick up plasmids with resistance traits, and the number of plasmid relics carrying resistance genes is high, as is the number of antibiotic gene combinations on those plasmids.37

Continuous surveillance to monitor this potential upcoming problem is important, as are prudent infection-control measures and antibiotic use to prevent and control the spread of these antibiotic-resistant strains. Maybe the preventive use of cranberry tablets or lactobacilli could decrease empiric antibiotic use.38,39

Conclusion

In this study, we observed a high prevalence of antibiotic resistance but a low prevalence of ESBL production among E. coli isolates collected from urine samples from NH residents. Antibiotic use was also relatively high. Particularly the use of and resistance to fluoroquinolones is a point of concern. Because of this prevalence of resistance, many antibiotics including amoxicillin-clavulanic acid, the folate antagonists, and the fluoroquinolones are no longer suitable for empiric treatment and, therefore, oral treatment options have become limited. Moreover, E. coli STs globally emerging as multidrug resistant strains have also been demonstrated in this study and pose a potential risk to this vulnerable population, because the risk for treatment failure would increase. We clearly demonstrated that the resistance among NH residents is different from elderly living at home and hospitalized patients, and that with the emergence of multidrug resistant strains worldwide, such as ST131, NHs are a potential reservoir for antibiotic (multidrug) resistance. Therefore, continuous surveillance of antimicrobial resistance in NHs is needed to monitor resistance over time and the increase of multidrug-resistant strains, and should be continued to be able to adapt treatment protocol if necessary. Also, antibiotic treatment should be initiated with care, and adequate infection-control measures should be applied.

Acknowledgments

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References


Table 3
Consumption of Antibiotics for Systemic Use in Defined Daily Dose/1000 Residents/Day

<table>
<thead>
<tr>
<th>ATC Group</th>
<th>Therapeutic Group</th>
<th>NH 1 (N = 130)</th>
<th>NH 2 (N = 180)</th>
<th>NH 3 (N = 329)</th>
<th>NH 4 (N = 282)</th>
<th>NH 5 (N = 104)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>J01AA</td>
<td>Tetracyclines</td>
<td>4.87</td>
<td>6.74</td>
<td>11.08</td>
<td>20.97</td>
<td>0.08</td>
<td>11.13</td>
</tr>
<tr>
<td>J01CA</td>
<td>Penicillins with extended spectrum</td>
<td>0.94</td>
<td>0.34</td>
<td>3.93</td>
<td>0.14</td>
<td>2.06</td>
<td>1.69</td>
</tr>
<tr>
<td>J01CE</td>
<td>Beta-lactamase sensitive penicillins</td>
<td>4.68</td>
<td>2.99</td>
<td>0.12</td>
<td>0.00</td>
<td>0.33</td>
<td>1.19</td>
</tr>
<tr>
<td>J01CF</td>
<td>Beta-lactamase–resistant penicillins</td>
<td>6.94</td>
<td>1.07</td>
<td>2.27</td>
<td>0.51</td>
<td>0.70</td>
<td>2.01</td>
</tr>
<tr>
<td>J01CR</td>
<td>Combination of penicillins including beta-lactamase inhibitors</td>
<td>19.86</td>
<td>17.50</td>
<td>18.01</td>
<td>20.59</td>
<td>38.28</td>
<td>20.92</td>
</tr>
<tr>
<td>J01DC</td>
<td>Second-generation cephalosporins</td>
<td>3.31</td>
<td>2.05</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.78</td>
</tr>
<tr>
<td>J01DD</td>
<td>Third-generation cephalosporins</td>
<td>0.00</td>
<td>0.00</td>
<td>0.12</td>
<td>1.84</td>
<td>1.52</td>
<td>0.67</td>
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<tr>
<td>J01EA</td>
<td>Trimethoprim and derivates</td>
<td>3.98</td>
<td>1.98</td>
<td>3.24</td>
<td>0.99</td>
<td>11.74</td>
<td>2.53</td>
</tr>
<tr>
<td>J01EE</td>
<td>Combinations of sulfonamides and trimethoprim including derivates</td>
<td>3.90</td>
<td>1.61</td>
<td>0.83</td>
<td>1.28</td>
<td>0.00</td>
<td>1.40</td>
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<tr>
<td>J01FA</td>
<td>Macrolides</td>
<td>0.00</td>
<td>0.34</td>
<td>0.23</td>
<td>1.38</td>
<td>0.49</td>
<td>0.56</td>
</tr>
<tr>
<td>J01FF</td>
<td>Lincosamides</td>
<td>0.20</td>
<td>1.48</td>
<td>0.67</td>
<td>3.06</td>
<td>2.13</td>
<td>1.56</td>
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<tr>
<td>J01MA</td>
<td>Fluoroquinolones</td>
<td>9.83</td>
<td>14.25</td>
<td>10.61</td>
<td>23.73</td>
<td>12.00</td>
<td>14.80</td>
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<td>J01FA</td>
<td>Nitrofuran derivates</td>
<td>2.50</td>
<td>9.82</td>
<td>1.59</td>
<td>4.47</td>
<td>12.22</td>
<td>5.02</td>
</tr>
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<td>J01</td>
<td>Antibacterials for systemic use</td>
<td>59.02</td>
<td>58.94</td>
<td>52.81</td>
<td>77.60</td>
<td>81.56</td>
<td>64.41</td>
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