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Carriage of antimicrobial-resistant commensal bacteria in Dutch long-term-care facilities

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Objectives: The objective of this study was to assess carriage of antimicrobial-resistant commensal microorganisms, i.e. *Escherichia coli* and *Staphylococcus aureus*, and its predictors in long-term-care facilities (LTCFs).

Methods: Nasal swabs and/or urine or incontinence samples were collected from participating residents in 111 LTCFs and tested for the presence of *S. aureus* and/or *E. coli*, respectively. Antimicrobial resistance to eight antimicrobials was linked to antimicrobial usage in the year preceding sampling and to LTCF characteristics. Using multilevel logistic regression, predictors of carriage of ESBL-producing *E. coli* in LTCFs were identified.

Results: *S. aureus* was identified in 1269/4763 (26.6%) nasal swabs, including 13/4763 (0.3%) MRSA carriers in 9/107 (8%) LTCFs. Of the 5359 urine/incontinence samples, 2934 (55%) yielded *E. coli*, including 123 (4.2%) producing ESBL, which were found in 53/107 locations (range 1%–33%). For all but one antimicrobial (i.e. nitrofurantoin) >20% of isolated *E. coli* were resistant. Multilevel multivariable logistic regression identified two predictors of carriage of ESBL-producing *E. coli*: (i) antimicrobial usage (OR 1.8, 95% CI 1.1–3.0 for each extra 50 DDD/1000 residents/day); and (ii) presence of MRSA carriers in the LTCFs (OR 2.4, 95% CI 1.0–5.6).

Conclusions: The low proportion of 4.2% ESBL-producing *E. coli* and the low prevalence of 0.3% MRSA carriage found in LTCF residents suggest that Dutch LTCFs are not yet an important reservoir of MDR potential pathogens. Nevertheless, the large variation between LTCFs warrants close monitoring of antimicrobial resistance in LTCFs. Integrated surveillance, i.e. linking data sources on antimicrobial usage, microbiological testing, clinical background data and epidemiological data, is needed.

Introduction

Antimicrobial resistance has become a significant health problem worldwide. Currently, the occurrence of infections due to MDR microorganisms is still rare in the Netherlands and other Northern European countries[1] and many efforts are in place to maintain this low-endemic situation. In the Netherlands, national guidelines have been developed for the prevention[2] and detection[3] of highly resistant microorganisms (HRMOs). In these guidelines, HRMOs are defined as 'microorganisms that are known to cause human disease, have acquired an antimicrobial resistance pattern that hampers (empirical) therapy, and have the potential to spread in healthcare facilities if—in addition to standard precautions—no transmission-based precautions are taken'. Since antimicrobial use is considered one of the most important factors selecting for antimicrobial resistance,[4] the aim should be to optimize the use of antimicrobials. Emergence of HRMOs in a low-endemic population may result in a reservoir from which transmission can occur, with potential consequences for vulnerable populations.[5] The elderly, especially those living in long-term-care facilities (LTCFs), are considered vulnerable to the acquisition of infections.[6] Not only is this population more susceptible to infections due to immunosenescence, but it is also a population often characterized by chronic diseases and polypharmacy, including antimicrobials.[7]

In healthcare settings, optimal (empirical) therapy requires up-to-date information concerning the local situation of antimicrobial resistance obtained by microbiological analysis of patient samples. Nevertheless, microbiological analysis of patient samples in cases of suspected infection is not common practice in LTCFs. To date, little is known about the occurrence of resistant microorganisms in Dutch LTCFs. Some relatively small regional studies were performed between 2010 and 2012.[8–10] These studies suggest a high local prevalence of antimicrobial-resistant *Escherichia coli*, including those producing an ESBL. Studies performed in 2009 covering a larger part of the Netherlands found low prevalence of 0.3%[11] and 2%[12] of MRSA in Dutch LTCFs.

Here, we investigate the carriage of antimicrobial-resistant commensal flora in Dutch LTCF residents, to assess whether LTCFs may act as a reservoir of antimicrobial-resistant potential pathogens. To find predictors of carriage of resistant microorganisms, we linked the observed antimicrobial resistance to antimicrobial usage and to LTCF characteristics. Two microorganisms were included, i.e. *E. coli* and *Staphylococcus aureus*, the most common causative agents[13] of frequently occurring infections in LTCF residents: urinary tract and skin infections.[14]

Methods

Selection of LTCFs

All 177 LTCFs in the Netherlands with at least 50 beds (excluding rehabilitation) were invited to participate. Enrolment of individual residents occurred by informed consent during the first months of the study, and by an opt-out approach later on, in order to increase the response rate among residents within each LTCF. If the client was unable to provide informed consent or to agree to the opt-out approach, e.g. due to dementia, the legally responsible person was asked for consent to participate.

A sample size calculation to enable determining a prevalence of 2% ESBL-producing *E. coli*, with a 95% CI of 1%–3%, indicated the need for a minimum of 40 nursing homes with participation of a minimum of 40 residents.

Data collection

All patient samples were collected anonymously; only gender was registered.

Nasal swabs (Copan cotton swabs including transport medium) were taken from the resident's anterior nostril by the members of the project team and analysed for the presence of *S. aureus* using: (i) culture on colistin nalidixic acid agar plates (Becton Dickinson, Sparks, MD, USA); and (ii) enrichment in nutrient broth (Oxoid, Basingstoke, UK) with 6.5% NaCl followed by culture on oxacillin screening agar (Oxoid). Putative *S. aureus* colonies were identified using MALDI-TOF. Methicillin resistance was confirmed by PCR as previously described.[15] *S. aureus* isolates resistant to oxacillin and cefoxitin were analysed for the presence of the *mecA* gene by PCR.[16]

Urine samples were collected from asymptomatic residents by the nursing staff. Clean voided urine samples were used to inoculate a dipslide (Uriline, bioMérieux 56508). Each dipslide has a two-sided paddle in a protective vial with a cystine lactose electrolyte deficient (CLED) agar on one side and on the other side MacConkey agar. In case of urine incontinence, the dipslide was inoculated by pressing the paddle into the incontinence pad. After inoculation the dipslides were sent to the microbiological laboratory of the Maastricht University Medical Center (MUMC) and in the later part of the study to the National Institute of Public Health and the Environment (RIVM) and incubated overnight at 35°C.

Colonies grown on the dipslide were streaked onto a chromID plate (bioMérieux 43481) and incubated overnight. Putative *E. coli* were identified using MALDI-TOF. Putative ESBL-producing *E. coli* isolates were phenotypically confirmed using the Etest (bioMérieux) according to the guidelines of the Dutch Society for Medical Microbiology; the antibacterials used were ceftazidime, cefotaxime and cefepime with and without clavulanic acid. A difference in MIC ratio of $\geq$8 for one of these tested antimicrobial combinations was considered to indicate ESBL positivity. Antimicrobial susceptibility testing was performed using a microbroth dilution method[9] for doxycycline, amoxicillin, amoxicillin/clavulanate, trimethoprim, co-trimoxazole, ciprofloxacin, norfloxacin and nitrofurantoin. The EUCAST guidelines version 4.0 (http://www.eucast.org/clinical_breakpoints/, accessed 19 February 2015) were used for the susceptibility breakpoints. *E. coli* ATCC 25922 and ATCC 35218 were used as control strains.

Data on antimicrobial usage at LTCF level during the year preceding sampling within each LTCF (or group of LTCFs) were collected from the pharmacy linked to the participating locations. Details of collection of usage data are described by M. Roukens, L. Verhoef, E. Stobberingh and S. Natsch (unpublished data). Data are presented as DDD per 1000 residents per day for each LTCF, assuming 100% bed utilization capacity.[17] If data were only available for a group of LTCFs, i.e. an 'LTCF cluster', the DDD/1000 residents/day was based on the cluster's cumulative number of beds and all cluster locations were attributed the same DDD/1000 residents/day.

All participating locations received a standardized questionnaire addressing general characteristics of each LTCF.

Data analysis

One *E. coli* isolate was obtained per sample. For the total number of *E. coli* isolates, we calculated: (i) the 'sample prevalence' of *E. coli*, calculated as the number of *E. coli* isolated divided by the number of samples taken; (ii) the 'isolate prevalence' of resistant *E. coli* to each of the eight antimicrobials tested, as the number of resistant *E. coli* per antimicrobial divided by the total number of *E. coli* isolated; and (iii) the 'isolate prevalence' of ESBL-positive *E. coli*, as the number of ESBL-producing *E. coli* divided by the total number of *E. coli* isolated. For MRSA we calculated: (i) the 'sample prevalence' of MRSA carriers among residents, i.e. the number of MRSA-positive nasal swabs divided by the total number of nasal swabs taken; and (ii) the 'isolate prevalence' of MRSA as the total number of MRSA isolated divided by the number of *S. aureus*. Prevalences were calculated as totals for all participating LTCFs as well as for each LTCF separately. The mean prevalences were weighted using the 'survey' package in R version 3.20,[18] enabling accounting for the proportion sampled and potential cluster effect of sampling within the finite population of LTCFs in the Netherlands. For each LTCF, antimicrobial usage in DDD/1000 residents/day was compared with the isolate prevalence of resistance to each of eight tested antimicrobials, using Pearson's correlation coefficient if appropriate, with weighting for the proportion of total number of residents sampled.

For all LTCFs for which a complete dataset was available, univariable logistic regression analysis was performed to identify factors associated with the isolate prevalence of ESBL-producing *E. coli*. The 'glimmix' procedure in SAS for Windows version 9.3 (SAS institute Inc., USA) was used for multilevel univariable and multivariable logistic regression. A random intercept was included for groups of LTCFs (LTCF clusters) as a correction factor for a possible cluster effect of locations belonging to the same organization. Factors with P values <0.2 in univariable logistic regression were included in multivariable logistic regression using backward selection. If multiple variables for one characteristic showed a P value <0.2, the variable with the strongest predictor was selected. If statistically significant ($P<0.05$), predictors remained in a multivariable model. The final model was checked for confounders and analysed in strata for potential modifiers, and assessed for goodness of fit. Factors related to study design, i.e. geographical region and study period, showing significant associations were considered confounders if they altered the OR in the final model by $\geq$10%.

Ethics

The medical ethics committee of the MUMC approved the study design (METC 12-4-056).

Results

Of 177 LTCFs approached for participation, 111 (63%) were interested in participation and visited for sampling between October 2012 and July 2014 (Figure 1). Reasons for no participation

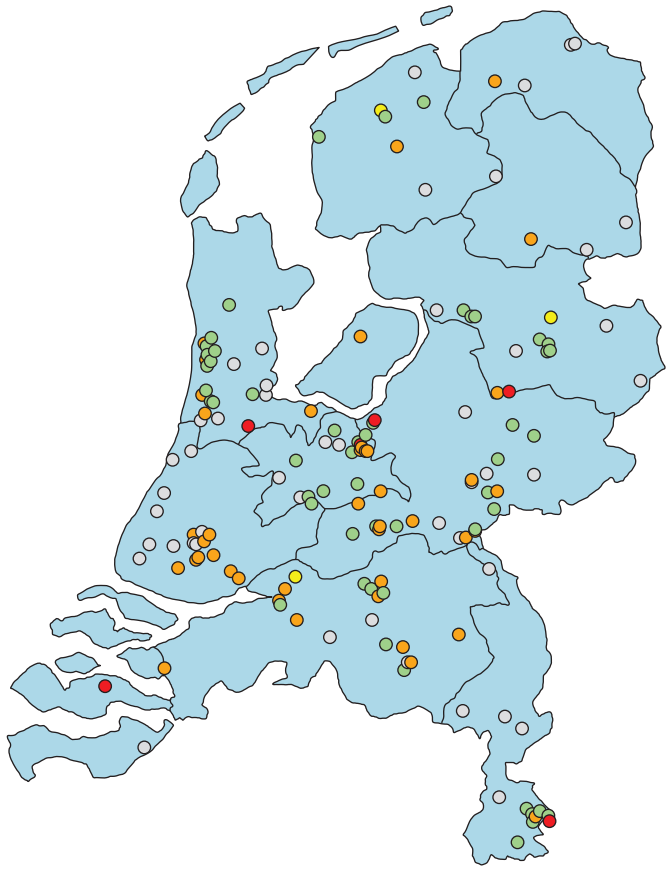

Figure 1. Geographical representation of LTCFs in the Netherlands approached for this study. The coloured dots represent the LTCFs included in this study. The grey dots represent LTCFs that chose not to participate. This figure appears in colour in the online version of *JAC* and in black and white in the print version of *JAC*.

were, among others, shortage of personnel and/or involvement in other studies.

Data collection

Urine samples were collected from 107 and nose swab samples from 107 of the 111 locations. A total of 96 of the 111 LTCFs (86%) provided data on antimicrobial usage and 80 of the 111 LTCFs (72%) provided the questionnaire data. A total of 64 of 111 (58%) participating locations provided complete data (Figure 2).

S. aureus was isolated in 1269 of 4763 (26.6%) nasal swabs, of which 13/1269 (isolate prevalence 1.0%) were MRSA, and thus 13/4763 (sample prevalence 0.3%) were MRSA carriers. MRSA was found in 9 of 107 (8%) locations tested, ranging from one to two carriers per positive location. Of the total number of dipslides taken ($N=5421$), 62 samples were excluded due to absence of growth or to contamination. Of these 5359 dipslides, 2934 (sample prevalence 55%) yielded *E. coli*. The isolate prevalence of resistance to the eight tested antimicrobials was ≥20% for all but one antimicrobial (nitrofurantoin) (Table 1). Of 2934 *E. coli* isolates, 123 (isolate prevalence 4.2%) produced ESBL. These were present in 53 of 107 (50%) locations tested. The isolate prevalences in LTCFs ranged from 1% to 33% (median 6.5%).

Antimicrobial usage and its association with isolate prevalence of ESBL-producing E. coli

For 53 LTCF locations, belonging to 11 LTCF clusters of locations belonging to the same nursing home group, data on use of antimicrobials were available at aggregated level, whereas for the remaining 43 locations, belonging to 24 clusters, data were available for each LTCF location separately. Of the 96 LTCFs with data on usage available, 92 were also tested for *E. coli* resistance. The mean total usage of antimicrobials for systemic use in these 92 LTCFs was 66.5 DDD/1000 residents/day, varying between LTCF locations from 1.8 to 197.5 DDD/1000 residents/day. Means and ranges of use of the eight antimicrobials tested are presented in Table 1. Amoxicillin/clavulanate was most frequently prescribed. Twenty-five percent of *E. coli* were resistant to this antimicrobial agent. The use of amoxicillin/clavulanate showed a statistically significant positive association with the isolate prevalence of resistance to this agent (correlation coefficient of 0.25, $P=0.02$) (Table 1). Nitrofurantoin was the second most prescribed, but the isolate prevalence of resistance to this agent was much lower, i.e. 1%.

LTCF characteristics and possible predictors of carriage of ESBL-producing E. coli

Table 2 shows the usage and LTCF characteristics of the 64 participating locations with complete data. Multilevel univariable logistic regression for 80 possible predictors of isolate prevalence of ESBL-producing *E. coli* showed statistically significant association for eight characteristics (Table 3). In multilevel multivariable logistic regression, two predictors remained in the model as strongest predictors: (i) each extra 50 DDD/1000 residents/day nearly doubled (OR 1.8, 95% CI 1.1–3.0) the chance of an ESBL-producing *E. coli*; and (ii) the presence of MRSA carriers in the LTCFs more than doubled the likelihood of finding ESBL-producing *E. coli* (OR 2.4, 95% CI 1.0–5.6). Geographical region remained in the model as a confounder; no significant effect modification was found.

Discussion

The low isolate prevalence of 4.2% ESBL-producing *E. coli* and the low sample prevalence of MRSA of 0.3% found in LTCF residents, combined with the size of our study, suggest that Dutch LTCFs are not yet an important reservoir of MDR potential pathogens. The MRSA prevalence is of the same order of magnitude as those described in previous studies.[11,19] These results indicate a consistently low MRSA prevalence over the last decade. Similar results were found in Germany, where no increase in MRSA was

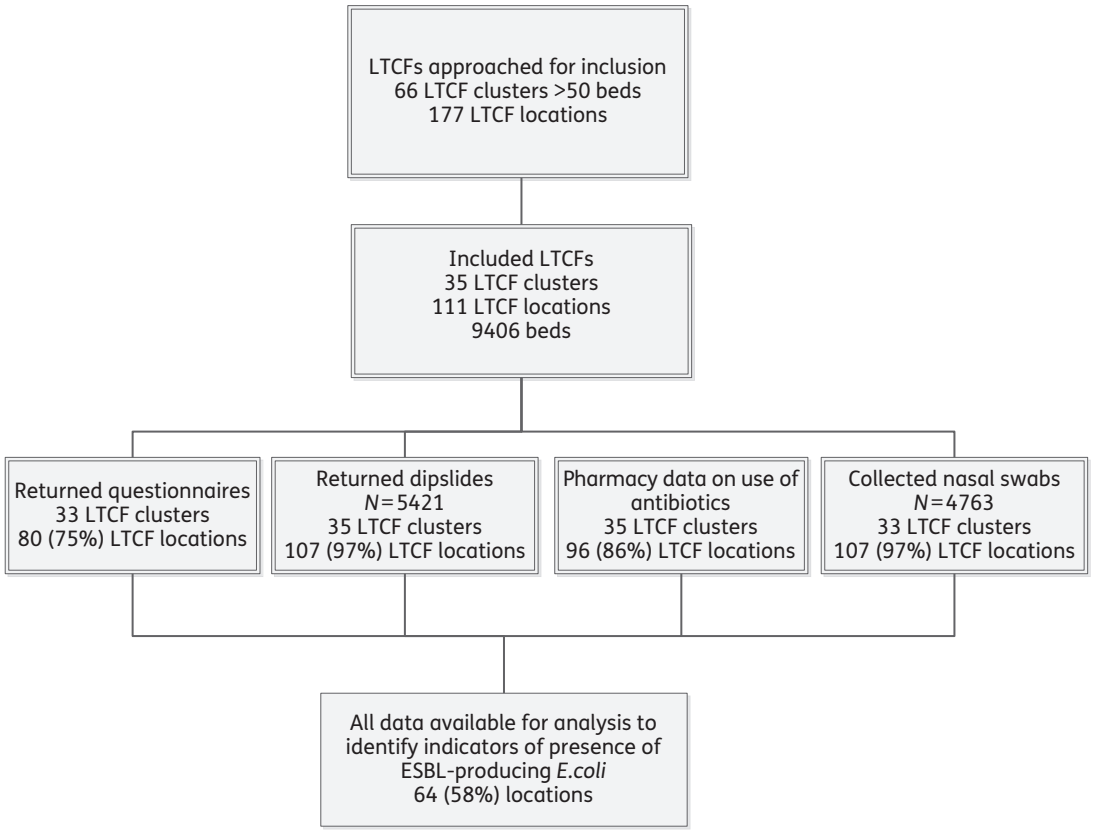

Figure 2. Selection of LTCFs and collection of questionnaire, laboratory and pharmacy data.

Table 1. The mean use correlated with weighted resistance levels of 2934 *E. coli* from 92 LTCFs for which both *E. coli* resistance and antimicrobial usage data were available, accounting for the number of residents sampled

Antimicrobial group	Antimicrobial agent	Mean usage[a] (95% CI)	Range of usage	Percentage of resistant *E. coli*[b]	Correlation coefficient
Tetracyclines	doxycycline	6.4 (5.2–7.6)	0.0–31.5	25 (22–27)	−0.07
β-Lactams	amoxicillin	4.2 (3.4–4.9)	0.0–19.1	45 (42–47)	−0.12
	amoxicillin/clavulanate	21.8 (18.8–24.9)	0.0–70.3	25 (23–28)	0.25[c]
Trimethoprim and derivatives	trimethoprim	1.7 (1.5–2.2)	0.0–13.9	25 (23–27)	−0.19
Sulphonamides	co-trimoxazole	2.7 (2.2–3.3)	0.0–12.6	22 (20–24)	0.18
Quinolones	ciprofloxacin	8.8 (7.1–10.6)	0.0–52.0	20 (18–23)	0.13
	norfloxacin	1.8 (1.3–2.2)	0.0–11.4	25 (22–27)	−0.17
Nitrofuran derivatives	nitrofurantoin	9.6 (7.8–11.3)	0.1–37.9	1 (0.6–1.6)	0.11

[a]In DDD/1000 residents/day.
[b]Susceptibility breakpoints for resistance: >0.5 for ciprofloxacin and norfloxacin; >2 for trimethoprim and co-trimoxazole; >4 for doxycycline; >8 for amoxicillin and amoxicillin/clavulanate; and >64 mg/L for nitrofurantoin.
[c]Statistically significant correlation ($P < 0.05$).

seen in LTCFs between 2007 and 2013,[20] and in Sweden between 2003 and 2012.[21]

Isolate prevalence of ESBL-producing *E. coli* showed a large variation between LTCFs: from 1% to 33%. An explanation for these variations in *E. coli* resistance levels was a higher usage of antimicrobials, which contributes to the selection of resistant *E. coli*. We also found that isolate prevalence of ESBL-producing *E. coli* was associated with the presence of MRSA. A causal

Table 2. Main characteristics of the 64/111 LTCFs included in the study and for which data were complete [i.e. urine (incontinence) samples, nose swabs, questionnaire, usage data]

	N or %	95% CI
Study design-related characteristics		
mean number of beds	83	68–98
mean number of urine (incontinence) samples collected	50	39–61
mean number of nose swabs collected	45	36–54
sampled in study period: 1/2	24/40	
region: North/East/South/West	2/23/9/30	
within 20 km from German and Belgian border: no (reference)/yes	59/5	
Overall resistance and usage		
weighted percentage of ESBL-producing *E. coli*	4.2%	3.3%–6.0%
weighted prevalence of MRSA	0.3%	0.2%–0.5%
mean total usage of antimicrobials in DDD/1000 residents/day	65	55–75
LTCF characteristics		
mean proportion of male residents	34%	30%–39%
mean number of staff (13 missing)	174	124–225
mean number of nursing staff (9 missing)	122	88–156
personnel exchange between care unit: no (reference)/yes (2 missing)	7/55	
percentage of residents with private room: ≤50% (reference)/>50% (1 missing)	19/44	
percentage of residents with private bathroom: ≤50% (reference)/>50% (2 missing)	38/24	
number of shared areas: ≤2 (reference)/>2 (6 missing)	28/30	
mean number of care units (range 1–22) (4 missing)	6	5–7
mean percentage of care packages		
4: physical disorders	2%	1%–3%
5: dementia	49%	41%–56%
6: chronic illness	22%	16%–28%
7: severe dementia	16%	13%–19%
8: illness for which specialized care is needed	7%	4%–11%
9: revalidation	3%	1%–5%
10: palliative care for terminal patients	0%	0%–1%
mean proportions of age groups of residents (5 missing)		
<60	4%	1%–7%
60–64	5%	2%–7%
65–74	9%	6%–11%
75–84	40%	33%–46%
≥85	43%	36%–50%
infection prevention committee: present/absent (2 missing)	51/11	
antimicrobial or medication committee: present/absent (6 missing)	46/12	
antimicrobial formularium: present/absent (12 missing)	37/15	
diagnostic tests available for residents		
leucocyte test: yes/no	52/12	
nitrite test: yes/no	49/15	
dipslide: yes/no	16/48	
culture: yes/no	48/16	

relationship is not likely; however, the association found may indicate the potential for successful resistance selection within these LTCFs, e.g. higher antimicrobial pressure, or fewer handwash facilities in place.

To our knowledge, this is the first large sample study with an integrated approach combining data on antimicrobial resistance, usage and LTCF characteristics. Despite a non-response of residents in LTCFs of nearly 50% for nasal swabs and >40% for urine sampling, this study still represents a relatively high proportion of the target population. The integrated approach at this large scale could be achieved through collection of data at the LTCF level instead of the patient level. In addition, the choice of urine samples instead of faecal samples and the change from informed consent to opt-out enabled us to obtain a sufficient response of residents per LTCF. Previous studies focused on patient-based risk factors only and found risk factors such as, among others, antimicrobial treatment during the past month, hospitalization during the past half year,[21] immobility, urinary

Table 3. Results of multilevel univariable and multivariable logistic (backward selection) regression analysis to find factors associated with presence or absence of ESBL-producing *E. coli* among residents in 64 LTCFs with complete data

Variable	OR (95% CI) univariable[a]	OR (95% CI) multivariable[b]
Number of shared areas: ≤2 (reference) versus >2	2.0 (1.0–3.9)	
Number of care units: 1–22[c]	0.7 (0.4–1.1)	
Proportion of residents with insurance package 5 or 7 (dementia)	0.2 (0.1–0.6)	
Percentage of residents with private room: ≤50% (reference) versus >50%	1.6 (0.9–2.8)	
Total usage of antimicrobials in DDD/1000 residents/day[d]	1.9 (1.2–3.0)	2.3 (1.5–3.6)
Within 20 km from German and Belgian border: no (reference) versus yes	0.1 (0.0–1.2)	0.0 (0.0–0.4)
MRSA present in LTCFs: no (reference) versus yes	2.7 (1.1–6.4)	2.4 (1.1–5.5)
Study period: first (reference) versus second	3.7 (1.2–11.8)	

[a]The following items were analysed in univariable analysis:
LTCF characteristics: proportion of males, number of beds, number of staff, number of nursing staff, number of public or shared areas, number of departments, proportion of patients categorized in insurance packages 4–10 [i.e. high level of care needed due to physical disorders (package 4); increased or high level of care needed due to psychological disorders (packages 5 and 7); highly specialized care needed (package 8); long-term care needed due to chronic disorders (package 6); revalidation (package 9); palliative care for terminal patients (package 10)], exchange of staff between departments, proportion of residents with own room/bathroom/toilet, proportion of residents in age categories (<60, 60–64, 65–74, 75–84 and ≥85 years of age), presence of an infection committee, presence of an antimicrobial or medicine committee, use of an antimicrobial formularium and use of diagnostic tests for residents with symptoms of urinary tract infection (leucocyte test, nitrite test, dipslide, culture or combinations of these tests).
Usage: use of antimicrobials for systemic use in total (J01) as well as in antimicrobial groups and antimicrobials tested, as presented in Table 1.
Study characteristics: study phase, received questionnaires, received pharmacy data and proportion of all residents sampled.
Geographical characteristics: provinces grouped in one variable as well as per province separately, location of LTCFs within 20 km from German border or 20 km from Belgian border or within 20 km from both the German and Belgian border and Northern versus Southern region of the Netherlands as well as North/East/South/West and North-Mid-South.
Categorization of variables was done based on the value representing 25th, 50th and/or 75th percentile among LTCFs where no ESBL-producing *E. coli* were observed.
[b]Selected as the strongest predicting representative of its category: total use of antimicrobials was chosen instead of each of the antimicrobials separately or antimicrobials in groups; region South versus North performed better than the three or four regions, province-based variables or border-based variables.
[c]OR is calculated per 5 care units.
[d]OR is calculated per 50 DDD/1000 residents/day.

catheter, decubitus,[22] the need for nursing care[23] and exposure to quinolones and third-/fourth-generation cephalosporins.[24] Here, we also take into account collective factors at the LTCF level, including antimicrobial pressure, and LTCF characteristics. We found that the total amount of antimicrobials used during the preceding year holds up as the strongest predictor of higher isolate prevalence of ESBL-producing *E. coli*, while correcting for LTCF characteristics. Inclusion of faecal samples in future studies to identify actual carriers of ESBL-producing *E. coli* would be of benefit.

Unfortunately data for 31 LTCF questionnaires were missing. Extrapolation of questionnaire data from one location to other locations belonging to the same LTCF cluster was not justified, as the characteristics of the different locations were not necessarily similar, based on comparison of the questionnaires of LTCF clusters for which multiple locations returned a questionnaire. However, LTCFs with missing questionnaires did not differ statistically significantly in their ESBL-producing *E. coli* isolate prevalence. The same was true for LTCFs with and without antimicrobial usage data available. So the non-response in questionnaire and usage data was not likely to result in bias with respect to the ESBL-producing *E. coli*. Study period and geographical location are factors that may be associated with the study design. In the first phase of the study, the chance of detecting ESBL-producing *E. coli* was smaller compared with the second phase of the study due to higher numbers of samples collected per LTCF location in the second, opt-out phase. The geographical location categorized in '<20 versus ≥20 km from one of the borders' appeared to be the strongest predictor; however, only few LTCFs in the border region were included. Therefore, both design-related significant factors were considered potential confounders. The regional distribution seemed skewed to the west and to be underrepresented for the northern part of the Netherlands, which was consistent with population densities in these areas.

Our study provides insight into the sample and isolate prevalence of carriage of resistant microorganisms by residents of LTCFs in the Netherlands. Once vulnerable, a carrier might turn into a severely ill patient. If an isolate prevalence >20%[25] is assumed as the cut-off above which the antimicrobial is no longer regarded as an empirical choice of treatment, our results suggest that only nitrofurantoin remains as a first-choice option out of the eight tested antimicrobials. The results of our study were provided as feedback to the participating LTCFs in order to support prudent and appropriate use of antimicrobials.

Although levels of carriage of resistant microorganisms are still low in the Netherlands, we show that a higher amount of antimicrobial used is associated with higher levels of carriage of *E. coli* resistant to antimicrobials in LTCFs. Previous studies by van Buul *et al.*[26] illustrated the difficulties in appropriate prescribing of antimicrobials in LTCFs, due to several unrelated factors affecting this

decision-making in LTCFs. Integrated surveillance, i.e. linking data sources on antimicrobial usage, microbiological testing, clinical background data and epidemiological data, would yield a wealth of information for regular analysis of predictors of carriage of resistant microorganisms and evaluation of the effects of intervention in LTCFs.

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References

1 Albu C, Ciancio B, Diaz Högberg L *et al*. *Annual Epidemiological Report 2014—Antimicrobial Resistance And Healthcare-Associated Infections*. Stockholm: ECDC, 2015.

2 WIP. *Verpleeghuizen, Woonzorgcentra En Voorzieningen Voor Kleinschalig Wonen Voor Ouderen*. 2014. http://www.rivm.nl/dsresource?objectid=rivmp:261540&type=org&disposition=inline.

3 Kluytmans-Vandenbergh MF, Kluytmans JA, Voss A. Dutch guideline for preventing nosocomial transmission of highly resistant microorganisms (HRMO). *Infection* 2005; **33**: 309–13.

4 van Buul LW, van der Steen JT, Veenhuizen RB *et al*. Antibiotic use and resistance in long term care facilities. *J Am Med Dir Assoc* 2012; **13**: 568.e1–13.

5 Weterings V, Zhou K, Rossen JW *et al*. An outbreak of colistin-resistant *Klebsiella pneumoniae* carbapenemase-producing *Klebsiella pneumoniae* in the Netherlands (July to December 2013), with inter-institutional spread. *Eur J Clin Microbiol Infect Dis* 2015; **34**: 1647–55.

6 Nicolle LE, Strausbaugh LJ, Garibaldi RA. Infections and antibiotic resistance in nursing homes. *Clin Microbiol Rev* 1996; **9**: 1–17.

7 Kantor ED, Rehm CD, Haas JS *et al*. Trends in prescription drug use among adults in the United States from 1999-2012. *JAMA* 2015; **314**: 1818–31.

8 Hoogendoorn M, Smalbrugge M, Stobberingh EE *et al*. Prevalence of antibiotic resistance of the commensal flora in Dutch nursing homes. *J Am Med Dir Assoc* 2013; **14**: 336–9.

9 van der Donk CF, Schols JM, Driessen CJ *et al*. Prevalence and spread of multidrug resistant *Escherichia coli* isolates among nursing home residents in the southern part of The Netherlands. *J Am Med Dir Assoc* 2013; **14**: 199–203.

10 Platteel TN, Leverstein-van Hall MA, Cohen Stuart JW *et al*. Predicting carriage with extended-spectrum β-lactamase-producing bacteria at hospital admission: a cross-sectional study. *Clin Microbiol Infect* 2015; **21**: 141–6.

11 Greenland K, Rijnders MI, Mulders M *et al*. Low prevalence of methicillin-resistant *Staphylococcus aureus* in Dutch nursing homes. *J Am Geriatr Soc* 2011; **59**: 768–9.

12 van der Donk CF, Rijnders MI, Donker GA *et al*. Is living in a border region a risk for a high prevalence of resistance? *Eur J Clin Microbiol Infect Dis* 2013; **32**: 989–95.

13 De Vecchi E, Sitia S, Romano CL *et al*. Aetiology and antibiotic resistance patterns of urinary tract infections in the elderly: a 6-month study. *J Med Microbiol* 2013; **62**: 859–63.

14 Ruscher C, Kraus-Haas M, Nassauer A *et al*. [Healthcare-associated infections and antimicrobial use in long term care facilities (HALT-2): German results of the second European prevalence survey]. *Bundesgesundheitsblatt Gesundheitsforschung Gesundheitsschutz* 2015; **58**: 436–51.

15 van der Donk CF, Schols JM, Schneiders V *et al*. Antibiotic resistance, population structure and spread of *Staphylococcus aureus* in nursing homes in the Euregion Meuse-Rhine. *Eur J Clin Microbiol Infect Dis* 2013; **32**: 1483–9.

16 Donker GA, Deurenberg RH, Driessen C *et al*. The population structure of *Staphylococcus aureus* among general practice patients from The Netherlands. *Clin Microbiol Infect* 2009; **15**: 137–43.

17 WHO Collaborating Centre for Drug Statistics Methodology. *Guidelines For ATC Classification And DDD Assignments*. 2015. http://www.whocc.no/atc_ddd_publications/guidelines/.

18 *R: A Language and Environment for Statistical Computing. 3.20*. Vienna, Austria: The R Foundation, 2015. http://www.R-project.org/.

19 de Neeling AJ, Wannet WJ. Study shows low rate of transmission of MRSA in Dutch nursing homes. *Euro Surveill* 2003; **7**: pii=2349.

20 Hogardt M, Proba P, Mischler D *et al*. Current prevalence of multidrug-resistant organisms in long-term care facilities in the Rhine-Main district, Germany, 2013. *Euro Surveill* 2015; **20**: pii=21171.

21 Sundvall PD, Elm M, Gunnarsson R *et al*. Antimicrobial resistance in urinary pathogens among Swedish nursing home residents remains low: a cross-sectional study comparing antimicrobial resistance from 2003 to 2012. *BMC Geriatr* 2014; **14**: 30.

22 Gruber I, Heudorf U, Werner G *et al*. Multidrug-resistant bacteria in geriatric clinics, nursing homes, and ambulant care--prevalence and risk factors. *Int J Med Microbiol* 2013; **303**: 405–9.

23 Min L, Galecki A, Mody L. Functional disability and nursing resource use are predictive of antimicrobial resistance in nursing homes. *J Am Geriatr Soc* 2015; **63**: 659–66.

24 Mitchell SL, Shaffer ML, Loeb MB *et al*. Infection management and multidrug-resistant organisms in nursing home residents with advanced dementia. *JAMA Intern Med* 2014; **174**: 1660–7.

25 Gupta K, Hooton TM, Naber KG *et al*. International clinical practice guidelines for the treatment of acute uncomplicated cystitis and pyelonephritis in women: a 2010 update by the Infectious Diseases Society of America and the European Society for Microbiology and Infectious Diseases. *Clin Infect Dis* 2011; **52**: e103–20.

26 van Buul LW, Sikkens JJ, van Agtmael MA *et al*. Participatory action research in antimicrobial stewardship: a novel approach to improving antimicrobial prescribing in hospitals and long-term care facilities. *J Antimicrob Chemother* 2014; **69**: 1734–41.