Journal of Hospital Infection 103 (2019) 160–164

Available online at www.sciencedirect.com

Journal of Hospital Infection

journal homepage: www.elsevier.com/locate/jhin

Short report

Spread of ESBL-producing *Escherichia coli* in nursing home residents in Ireland and the Netherlands may reflect infrastructural differences

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ARTICLE INFO

Article history:
Received 4 March 2019
Accepted 1 May 2019
Available online 9 May 2019

Keywords:
Nursing homes
Long term care facility
Colonization
Multi-drug-resistant organisms
Extended-spectrum beta-lactamase-producing Enterobacteriaceae
Clostridium difficile

SUMMARY

A prevalence study in two nursing homes (one each in the Netherlands and Ireland) found four (11%) Dutch and six (9%) Irish residents colonized with 11 extended-spectrum beta-lactamase-producing *Escherichia coli*, 10 of which contained CTX-M-15. Four Dutch isolates, from three residents of the same ward, belonged to *E. coli* O25:H4, sequence type (ST) 131 and were part of the same cluster type by whole-genome sequencing. Four Irish residents on three different wards were colonized with an identical *E. coli* O89:H9, ST131, complex type 1478. Cross-transmission between three Irish wards may reflect differences in nursing home infrastructure, specifically communal areas and multi-bedded resident rooms.

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https://doi.org/10.1016/j.jhin.2019.05.003

Introduction

Nursing home residents have multiple risk factors for colonization with multi-drug-resistant organisms (MDROs), and are potential reservoirs for transmission [1]. Frequent contact between residents due to communal living, high frequency of healthcare contacts and factors that facilitate MDRO spread, such as incontinence, present additional opportunities for transmission. MDRO prevalence varies considerably in nursing homes, from 55% colonization with extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* and 3% vancomycin-resistant enterococci (VRE) colonization in Ireland [2], to 4.2% ESBL-producing *E. coli* colonization in the Netherlands [3]. *Clostridium difficile* colonization in nursing home residents also varies from 4% to 51% [4]. As such a prevalence study was undertaken of *C. difficile* and MDRO colonization [specifically VRE, ESBL and carbapenemase-producing Enterobacteriaceae (CPE)] in two nursing homes, one in the Netherlands and the other in Ireland, to identify characteristics associated with carriage and risk factors for cross-transmission.

Materials and methods

Full-time residents of two nursing homes under the governance of the investigators' hospitals and in the investigators' catchment areas, one in the Netherlands and one in Ireland, were invited to participate. Written informed consent was required from the resident or his/her proxy. The nursing homes were similar to previously studied nursing homes in terms of infrastructure and resident demographics [5]. The Dutch nursing home consisted of 131 beds in eight wards of various sizes (12–35 beds); the wards had single en-suite rooms, except for three double rooms for couples. All wards had a separate dining area and the nursing home had a large communal recreation and physiotherapy area. The Irish nursing home consisted of 100 beds in four identical 25-bed wards with one communal recreation and dining area. Each ward consisted of a mixture of single (N=17), double (N=2) and four-bedded (N=1) en-suite rooms.

Demographic and MDRO risk factor data (care load indicators, hospitalization, antibiotics, urinary catheter use, wounds, pressure sores, previous MDROs or *C. difficile* infection) were collected for each consenting resident using standardized definitions [5] in February 2017 (6–17 February in the Netherlands, 6–10 February in Ireland). A corresponding faecal specimen was collected, stored at 4°C, and processed for multi-drug-resistant Enterobacteriaceae, VRE and *C. difficile* within 72 h of arrival at the laboratory, and subsequently stored at -20°C.

Ethical approval was granted by the 'Medisch Ethische Toetsings Commissie' of Leiden University Medical Centre (No. P16.039) and the Beaumont Hospital Ethics (Medical Research) Committee.

Following national recommendations, Dutch faecal samples were enriched in 15 mL of tryptic soy broth and incubated for 18 h at 35°C prior to plating on ChromID ESBL, VRE agar and MacConkey tobramycin agar (bioMérieux, Marcy l'Etoile, France) for 48 h at 35°C. In Ireland, faecal samples were directly inoculated on identical agar plates. Isolates were identified by the BD Bruker matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) Biotyper (Microflex, Bruker Daltonics, Bremen, Germany). Antibiotic susceptibility testing was performed by VITEK2 (The Netherlands; Card N199, bioMérieux) or Becton Dickinson (BD) Phoenix automated AST system (Ireland; BD Diagnostics, Franklin Lakes, NJ, USA) using the European Committee of Antimicrobial Susceptibility Testing breakpoints. ESBL production was confirmed using the double disk method. Specimens were screened for the presence of CPE, and isolates with a meropenem minimum inhibitory concentration >0.25 mg/L (Etest, bioMérieux) were investigated by an in-house multiplex polymerase chain reaction (PCR) to detect KPC, VIM, NDM, OXA-48 and IMP. *C. difficile* was detected as described previously [6], and suspected colonies were tested by MALDI-TOF (Ireland) or an in-house glutamate dehydrogenase PCR (the Netherlands) [6].

Whole-genome sequence (WGS) analysis was performed to further characterize ESBL-producing *E. coli* isolates from both nursing homes at GenomeScan (Leiden, the Netherlands). Genome sequences were determined using the Illumina HiSeq 4000 platform (Illumina, San Diego, CA, USA) from DNA prepared by the QIAsymphony DSP Virus/Pathogen Midi Kit (Qiagen, Hilden, Germany) following the manufacturer's recommendations. Sequence libraries were prepared using NEBNext Ultra II DNA Library Prep Kit for 150-bp paired-end sequencing. All raw sequencing data were submitted to the European Nucleotide Archive under accession numbers ERR3151305–ERR3151315. Core-genome multi-locus sequence typing (cgMLST) was performed using SeqSphere+ Version 5.1.0 (Ridom GmbH, Münster, Germany). The number of targets for *E. coli* is 2513 with a cluster-alert distance of 10. A minimum spanning tree based on the generated complex types was created in SeqSphere and expanded by uploading seven known complete genomes of *E. coli* ST131 (accession numbers: CP021179, CP021454, NC022648, CP014316, CP006784, CP010876, HG941718). The web-based tools ResFinder and RGI/CARD were used to determine antibiotic resistance genes.

Statistical analysis was performed using SPSS 23.0 and STATA SE Version 15.1 (StataCorp, College Station, TX, USA). Numerical data were compared with an unpaired t-test. For categorical data, an odds ratio (OR) was calculated using logistic regression, and presented with the 95% confidence interval (CI). For statistical comparisons, $P<0.05$ was considered to indicate significance.

Results

Data and a corresponding faecal specimen were collected from 37/131 (57.8%) Dutch and 67/98 (77.9%) Irish residents (Table I). One Dutch resident had previous VRE colonization, and 27 Irish residents had previously been colonized with MDROs (17 meticillin-resistant *Staphylococcus aureus*, seven VRE, three ESBL-producing *E. coli*). Dutch residents were less likely to have received an antibiotic and to have been hospitalized in the preceding 6 months (OR 0.31, 95% CI 0.14–0.73 and OR 0.19, 95% CI 0.04–0.92, respectively) (Table I).

Four (11%) Dutch and six (9%) Irish residents were colonized with 11 ESBL-producing *E. coli*. One Dutch resident was colonized with two different isolates. Of the Dutch isolates, four derived from three residents on Ward R were phenotypically similar on antibiotic susceptibility testing, including resistance against tobramycin and ciprofloxacin. The fifth

isolate, from a resident on a different ward (Ward L), was also resistant to gentamicin and trimethoprim/sulfamethoxazole (TMP/SMX), with intermediate resistance to tobramycin and ciprofloxacin. Of the six Irish isolates, residents were located on three different wards (Wards B, C and H); five isolates were resistant to ciprofloxacin and TMP/SMX, and one resistant was resistant to gentamicin. Ten (five Dutch and five Irish) of the 11 MDRO isolates harboured CTX-M-15. No residents were colonized with CPE or *C. difficile*. No Dutch residents were colonized with VRE, in contrast to one Irish resident.

The four Dutch ESBL-producing *E. coli* isolates from Ward R were typed as *E. coli* serotype O25:H4, sequence type 131. cgMLST analysis showed that two isolates (from two residents) had identical complex types (CT) 1172, and two isolates from a third resident were closely related (CT 1480 and 1479) and belonged to the same cluster type (Figure 1). The fifth Dutch isolate, from a different ward (Ward L), was distinct (CT 1483). All six Irish isolates were typed as *E. coli* serotype O25:H4, sequence type 131. Four isolates from residents on three different wards (Wards B, C and H) were closely related, and belonged to CT 1478 and the same cluster type (Irish cluster, Figure 1). The two other Irish isolates, CT 2923 and CT 1487, were unrelated. Four of the seven epidemiologic unrelated *E. coli* ST131 from Europe [Denmark (two isolates), Germany, Austria], the USA [Minneapolis (two isolates)] and Australia, with complex type 3100, clustered together in one cluster type (Figure 1).

None of the following were significantly different for ESBL-colonized (N=10) vs ESBL-negative (N=94) residents: age (OR 1.04, 95% CI 0.93–1.15), mean length of residence (OR 0.90, 95% CI 0.61–1.33), previous MDRO (OR 1.60, 95% CI 0.18–15.09), residence in a single room (OR 0.46, 95% CI 0.12–1.77), recent hospitalization or antibiotic use (OR 0.54, 95% CI 0.06–4.55 and OR 1.21, 95% CI 0.32–4.58, respectively), disorientation, faecal incontinence, urinary catheter, pressure sore or other wounds (OR 0.76, 95% CI 0.18–3.16; OR 2.55, 95% CI 0.62–10.49; OR 1.06, 95% CI 0.21–5.4; OR 4.45, 95% CI 0.74–26.71; OR 1.70, 95% CI 0.32–9.02; respectively).

Discussion

This study found that the prevalence of ESBL-producing *E. coli* in Ireland (9%) was lower than has been reported previously [2], whereas the prevalence of 11% in the Netherlands is in line with previous reports [3,7]. No residents were colonized with CPE or *C. difficile* in either country, and only one (Irish) resident was colonized with VRE. The prevalence of antibiotic use in both nursing homes were similar to that reported previously [5]. No association was found between MDRO carriage and the investigated risk factors, which reflects the low number of MDRO-colonized residents.

Nine of the 11 (82%) ESBL-producing *E. coli* isolates belonged to MLST ST131, with CTX-M-15 as the most common ESBL. The predominance of ST131 is not surprising as it is associated with older age [8], and is frequently observed in European nursing homes [2,7]. Of the seven epidemiologically unrelated *E. coli* ST131 NCBI strains from Europe, the USA and Australia, four

Table I
Sociodemographics and risk factors for multi-drug-resistant organism (MDRO) and *Clostridium difficile* colonization and infection of residents in the Dutch (NL) and Irish (IR) nursing homes.

	NL (N=37)	IR (N=67)	NL+IR (N=104)	Odds ratio[a] (95% CI) (NL vs IR)
Mean no. of beds/room	1.1	1.8	1.5	
Single rooms	34 (91.9%)	44 (65.7%)	78 (75.0%)	**5.92 (1.64–21.39)**
Double rooms	3 (8.1%)	8 (11.9%)	11 (10.6%)	0.65 (0.16–2.61)
Four-bedded rooms	0 (0.0%)	15 (22.4%)	15 (14.4%)	-
Mean length of residence (range)	2.1 years (0.7–3.8)	2.9 years (0.05–5.98)	2.6 years	**0.036**[b]
Mean age (range)	84.5 years (66–95)	84.1 years (69–94)	84.2 years	0.771[b]
Gender: female (%)	25 (67.6%)	43 (64.2%)	68 (65.4%)	0.86 (0.37–2.01)
Mobility: ambulant	14 (37.8%)	22 (32.8%)	36 (34.6%)	1.25 (0.54–2.87)
Mobility: wheelchair	23 (62.2%)	39 (58.2%)	62 (59.6%)	1.17 (0.52–2.69)
Mobility: bed-ridden	0 (0.0%)	6 (9.0%)	6 (5.8%)	-
Disorientated	25 (67.6%)	53 (79.1%)	78 (75.0%)	0.55 (0.22–1.36)
Recent[c] hospitalization	2 (5.4%)	15 (22.4%)	17 (16.3%)	**0.19 (0.04–0.92)**
Current antibiotic use	3 (8.1%)	8 (11.9%)	11 (10.6%)	0.65 (0.16–2.61)
Recent[c] antibiotic use	14 (37.8%)	44 (65.7%)	58 (55.8%)	**0.31 (0.14–0.73)**
Urinary catheter *in situ*	3 (8.1%)	17 (25.4%)	20 (19.2%)	**0.26 (0.07–0.95)**
Pressure sore	5 (13.5%)	2 (3.0%)	7 (6.7%)	5.08 (0.93–27.62)
Other wounds	13 (35.1%)	1 (1.5%)	14 (13.5%)	**35.75 (4.43–288.14)**
Incontinence: urine	24 (64.9%)	38 (56.7%)	62 (59.6%)	1.21 (0.52–2.82)
Incontinence: faeces	7 (18.9%)	43 (64.2%)	50 (48.1%)	**0.11 (0.08–0.55)**
Incontinence: both	7 (18.9%)	33 (49.3%)	40 (38.5%)	0.21 (0.82–4.50)
Proton pump inhibitor use	26 (70.3%)	37 (55.2%)	63 (60.6%)	1.91 (0.82–4.50)

CI, confidence interval.
[a] Significance is indicated in bold. The risk factor analysis was only performed for the residents from whom faeces was collected.
[b] For age and length of stay, differences between the two countries were calculated with an unpaired t-test. Instead of an odds ratio, the P-value is shown.
[c] In the preceding 6 months.

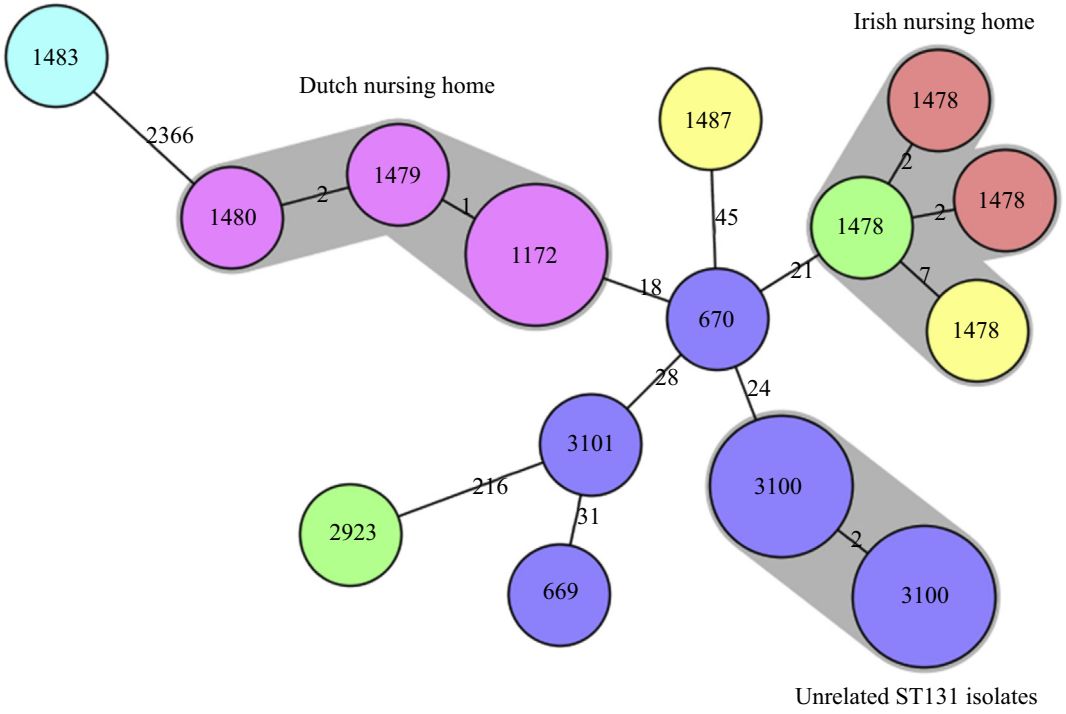

Figure 1. Minimum spanning tree of core genome multi-locus sequence typing (cgMLST) data of 11 extended-spectrum beta-lacatamase-producing *Escherichia coli* isolates. Circles represent a cgMLST complex type. The large circles represent two multi-drug-resistant organism (MDRO) isolates (complex types 1172 and 3100), and the small circles represent one isolate each. The circles are connected to the closest relative; the numbers on the connecting lines give the number of genes containing single nucleotide polymorphisms. Colours represent MDRO isolates from different wards in both nursing homes and the unrelated sequence type (ST) 131 isolates. In Ireland: Ward B in red, Ward C in yellow and Ward H in green. In the Netherlands, Ward R in pink and Ward L in turquoise. The unrelated ST131 isolates are coloured in purple. Zones around the circles indicate the presence of closely related isolates belonging to the same cluster type (cluster-alert distance: 10).

clustered together with cgMLST in one cluster type. This further underlines the clonality of this pandemic strain.

WGS analysis of *E. coli* isolates showed possible small-scale spread between three wards in the Irish nursing home and within one ward in the Dutch facility. There may have been more opportunities for cross-transmission in Ireland because of multi-bedded rooms and communal dining, in contrast to predominance of single rooms and ward-based dining in the Netherlands. Transmission of ESBL-producing Enterobacteriaceae is higher within households than in hospitals (23% vs 4.5, $P<0.01$), emphasizing faecal–oral transmission in ESBL epidemiology [9]. Likewise, a recent Dutch study reported co-carriage between preschool children and their parents within the same household with identical extended-spectrum cephalosporin-resistant Enterobacteriaceae, suggesting clonal transmission between children and parents within the household [10]. If transmission dynamics in nursing homes are reflective of household contact MDRO transmission, the consequences of colonization and initial small-scale MDRO spread could be significant. This would be compounded in nursing homes by faecal incontinence (64% Irish residents and 19% Dutch residents in this study), communal areas and multi-bedded resident rooms. In addition, a simulation study of MDRO transmission noted that while the daily probability of transmission in nursing homes was less than in the acute hospital setting, the longer length of resident stay (mean 2.6 years in this study) can facilitate cross-transmission; hence, hospital-based control efforts may not be effective in preventing nationwide outbreaks [1].

In this study, no residents were colonized with CPE or *C. difficile*, and only one Irish resident was colonized with VRE. This is in line with previous reports [2,4], although a higher prevalence of *C. difficile* colonization was reported in Ireland (10%), albeit in a single nursing home study.

Limitations of this study include its cross-sectional design, which was chosen for pragmatic reasons, potential selection bias from inclusion of a single nursing home per country, and low resident consent and specimen collection in the Netherlands, reflecting local challenges in acquiring informed consent but limiting the generalizability of findings. Specifically, the analysis of MDRO risk factors and association with MDRO colonization was underpowered because of low numbers, and the cross-sectional design limited analysis of epidemiological risk factors for colonization beyond associations with ward location. As data collection was based on previous European nursing home prevalence studies [5], additional data, such as scores for resident independency, that could impact on social contact with other residents were not collected. However, data on mobility (ambulant, wheelchair, bed-ridden) were collected as an indicator of care load, with little difference between the two care homes. Strengths of this study include the use of robust definitions, a standardized shared protocol and extensive molecular analysis. The study protocol was based on that from previous

European studies [5], and similar protocols for faeces collection and laboratory processing were employed. The only difference was the use of an MDRO enrichment broth in the Netherlands, which may have resulted in a higher recovery rate. However, both countries applied the national recommended culture methods, enabling national comparison, and previous Irish studies did not use an enrichment step, enabling comparison [2].

In conclusion, in a nursing home prevalence study, the high abundance of risk factors did not lead to high prevalence of MDROs. cgMLST analysis showed small-scale spread of MDROs between residents of the same ward in the Netherlands and on different wards in Ireland. This may reflect differences in nursing home infrastructure, specifically communal areas and multi-bedded resident rooms in the Irish nursing home which were not present in the Netherlands.

Acknowledgements

The authors wish to thank all participating nursing home residents, their families and the staff of both nursing homes: Woonzorgcentra Haaglanden, the Hague in the Netherlands and Raheny Community Nursing Unit, Dublin in Ireland. In addition, the authors would like to thank Ms Breffni Smith, Royal College of Surgeons in Ireland and Beaumont Hospital Library for assistance with the literature search.

Conflict of interest statement
None declared.

Funding source
Part of this research was made possible by an unrestricted grant of the National Institute of Public Health and the Environment, Centre for Infectious Disease Control in the Netherlands.

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