Exploring the role of the microbiota: in defence against Clostridioides difficile and multidrug resistant Gram-negatives

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Chapter 4

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

Low MDRO rate in nursing home residents

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Chapter 4. Spread of ESBL-producing *Escherichia coli* in nursing home residents in Ireland and the Netherlands may reflect infrastructural differences

Abstract

A prevalence study in two nursing homes (the Netherlands and Ireland) found four (11%) Dutch and six (9%) Irish residents colonised with 11 extended-spectrum ß-lactamase (ESBL)-producing *Escherichia coli*, ten containing 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 colonised 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.

Introduction

Nursing home residents have multiple risk factors for colonisation with multidrug resistant organisms (MDRO), and are potential reservoirs for transmission [1]. Frequent contact between residents due to communal living, high frequency of healthcare contact and factors that facilitate MDRO spread such as incontinence present additional opportunities for transmission. MDRO prevalence varies considerably in nursing homes from 55 % colonisation with extended spectrum beta-lactamase (ESBL)-producing *Escherichia coli* and 3 % vancomycin resistant enterococci (VRE) colonisation in Ireland [2], to 4.2 % ESBL-producing *E. coli* colonisation in the Netherlands [3]. *Clostridioides difficile* colonisation in nursing home residents also varies from 4 – 51 % [4]. We conducted a prevalence study of *C. difficile* and MDRO colonisation (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 area, one in the Netherlands and one in Ireland, were invited to participate. Written informed consent by the resident or his/her proxy was required. 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 varying in size (12 – 35 beds) that consisted of 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 common recreation and dining area. Each ward consisted of a mixture of single (n=17), double (n=2) and four-bed (n=1) en-suite rooms.

Demographic and MDRO risk factor data (care load indicators, hospitalisation, antibiotics, urinary catheter use, wounds, pressure sores, previous MDRO or CDI) were collected on 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 also collected, stored at 4°C, processed for multidrug-resistant Enterobacteriaceae, VRE and *C. difficile* within 72 hours of arrival to the laboratory and subsequently stored at -20°C.

Ethical approval was granted by the medical ethical committee "Medisch Ethische Toetsings Commissie" of Leiden University Medical Center (no. P16.039) and the Beaumont Hospital Ethics (Medical research) committee.

Following national recommendations, Dutch faeces samples were enriched in 15ml of Tryptic Soy Broth and incubated for 18 hours at 35°C prior to plating on ChromID ESBL, VRE agar and MacConkey tobramycin agar (bioMérieux, Marcy l'Etiole, France) for 48 hours at 35°C. In Ireland, faeces were directly inoculated on identical agar plates. Isolates were identified by the BD Bruker MALDI-TOF Biotyper (Microflex, Bruker Daltonics, Bremen, Germany). Antibiotic susceptibility testing was performed by VITEK2 (The Netherlands; card N199, bioMérieux) or BD Phoenix™ automated AST system (Ireland; BD Diagnostics), using the European Committee of Antimicrobial Susceptibility Testing breakpoints. ESBL production was confirmed by a 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) investigated by an in-house multiplex PCR to detect KPC, VIM, NDM, OXA-48 and IMP. *C. difficile* was detected as previously described **[6]**, and suspected colonies tested by MaldiTOF (Ireland) or an in-house GDH PCR (the Netherlands) **[6]**.

Whole genome sequence analysis (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) 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 a 150 bp paired-end sequencing. All raw sequencing data was submitted to the European Nucleotide Archive (ENA) under accession numbers ERR3151305 - ERR3151315. Core-genome Multi Locus Sequence Typing (cgMLST) was performed using SeqSphere+ software version 5.1.0 (Ridom GmbH). 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, Texas, US). Numerical data were compared with an unpaired t-test. For categorical data, an odds ratio (OR) was calculated using logistic regression and presented with a 95% confidence interval (95% CI). For statistical comparisons, a p-value below 0.05 was considered significant.

Results

Data and a corresponding faeces specimen was collected from 37/64 (57.8%) Dutch and 67/86 (77.9%) Irish residents. (Table 1). One Dutch resident had previous VRE colonisation, whereas 27 Irish residents were previously MDRO colonised (17 methicillin resistant *Staphylococcus aureus*, seven VRE, three ESBL-producing *E. coli*). Dutch residents were less likely to have received an antibiotic and be hospitalised in the previous six months (OR 0.31, CI 0.14-0.73 and OR 0.19, CI 0.04-0.92 respectively) (Table 1).

Table 1. Socio-demographics and risk factors for multidrug resistant organism (MDRO) and *C. difficile* colonisation 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	
Room type: Single	34 (91.9%)	44 (65.7%)	78 (75.0%)	5.92 (1.64 – 21.39)
Room type: Double	3 (8.1%)	8 (11.9%)	11 (10.6%)	0.65 (0.16 – 2.61)
Room type: Four-bed	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)
Recentc hospitalisation	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)
Recentc 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)

a Significance is indicated in bold. The risk factor analysis was only performed for the residents of which faeces was collected.
b For age and length of stay of the residents, differences between the two countries was calculated with an unpaired t-test. Instead of an odds ratio, the p-value is shown.
c In the previous six months
CI: Confidence Interval

Four (11%) Dutch and six (9%) Irish residents were colonised with eleven ESBL-producing *E. coli*. One Dutch resident was colonised 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, H); five isolates were also resistant to ciprofloxacin and TMP/SMX and one resistant to gentamicin. Ten (five Dutch and five Irish) of the eleven MDRO isolates harboured CTX-M-15. No residents were colonised with CPE or *C. difficile*. No Dutch residents were colonised 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. Core genome (cg) MLST analysis showed that two isolates (from two residents) had identical complex types (CT) 1172, two isolates from a third resident were closely related (CT 1480 and 1479) and belonged to the same cluster type (Fig 1). The fifth Dutch isolate from a different 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, 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), United States (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-colonised (n=10) versus ESBL-negative (n=94) residents; age (OR1.04, 95% CI 0.93 – 1.15), mean length of residence (OR 0.90 95% CI 0.61 – 1.33), previous MDRO (OR1.60 95% CI 0.18 – 15.09), residence in a single room (OR 0.46, 95% CI 0.12-1.77), recent hospitalisation 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], and OR 1.70, 95% CI [0.32, 9.02], respectively.

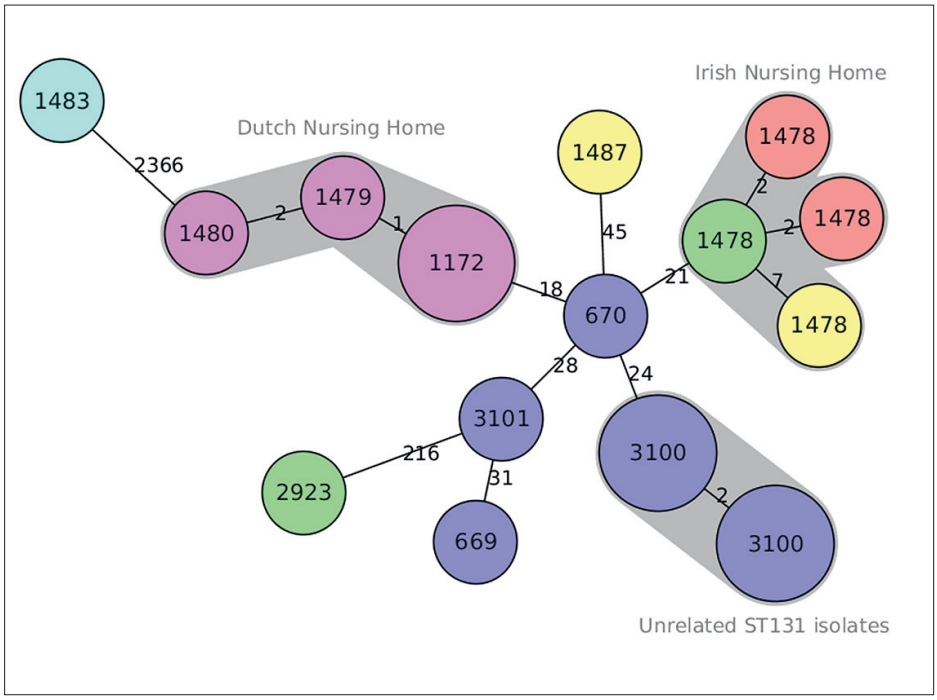

Figure 1. Minimum spanning tree of core genome MLST data of 11 ESBL-producing *E. coli* isolates

Circles: represent a core genome (cg) MLST complex type. A larger size circles represents two MDRO isolates (Complex Type 1172 and 3100), the smaller circles one isolate each. The circles are connected to the closest relative; the numbers on the connecting lines give the number of genes containing SNPs.

Colours: represent MDRO isolates from different wards in both nursing homes and the unrelated ST131 isolates. In Ireland; ward B in red, ward C in yellow, ward H in green. In the Netherlands; ward R in pink and ward L in turquoise. The unrelated ST131 isolates are coloured in blueberry blue.

Zones around the circles indicate the presence of closely related isolates belonging to the same cluster type (cluster alert distance: 10).

Discussion

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

Nine of the eleven (82 %) ESBL-producing *E. coli* isolates belonged to the MLST ST131 with *CTX-M-15* as most common ESBL. The predominance of ST131 is not surprising as it is associated with and 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 US and Australia, four clustered together with cgMLST in one cluster type. This further underlines the clonality of this pandemic strain.

WGS of *E. coli* isolates shows 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 was higher within households than in hospitals (23 % versus 4.5 for ESBL *E. coli*, $p < 0.01$) emphasising 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, then the consequences of colonisation and initial small-scale MDRO spread could be significant. This would be compounded in nursing homes by faecal incontinence (e.g., 64 % Irish versus 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 the acute hospital setting, the longer length

of resident stay (*e.g.* mean 2.6 years in our 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 colonised with CPE or *C. difficile*, and only one Irish resident was colonised with VRE, which is in line with previous reports [2, 4], although higher *C. difficile* colonisation prevalence 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 only one nursing home per country, and low resident consent and specimen collection specifically in the Netherlands, reflecting local challenges in acquiring informed consent but limiting the generalisability of findings. Specifically, the analysis of MDRO risk factors and association with MDRO colonisation was underpowered because of low numbers and the cross-sectional design limited analysis of epidemiological risk factors for colonisation, 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) was collected as an indicator of care load with little difference between both sites. Strengths include the use of robust definitions, a standardized shared protocol, and the 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 a 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 MDRO prevalence. Core genome MLST analysis showed small-scale MDRO spread between residents of the same ward in the Netherlands and on different wards in Ireland. This may reflect differences in nursing home infra-

structure, specifically communal areas and multi-bedded resident rooms in the Irish nursing home which were not present in the Netherlands.

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Conflicts of interest

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