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## Major Article

## Low prevalence of colonization with multidrug-resistant gram-negative bacteria in long-term care facilities in Graz, Austria

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## Key Words:

Elderly  
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**Background:** Residents in long-term care facilities (LTCFs) are increasingly found to be an important reservoir of multidrug-resistant gram-negative (MRGN) bacteria.

**Aims:** We aimed to determine colonization by MRGN bacteria over 6 months in LTCFs and geriatric wards in Graz, Austria, and to evaluate risk factors for such colonization.

**Methods:** During August 2015, we conducted a point-prevalence survey at LTCFs and geriatric wards of the Geriatric Health Centers of the City of Graz. Inguinal and perianal swabs were taken from 137 patients and screened for MRGN using standard procedures. Six months after the initial investigation all colonized patients were sampled again and use of antibiotics, hospital admissions, and mortality was registered. Genetic relatedness of MRGN bacteria was evaluated.

**Results:** We detected 12 patients harboring MRGN isolates (prevalence, 8.7%). Overall inguinal colonization was 5.1%. After 6 months, only 2 out of 12 patients were still colonized. Presence of a urinary catheter was associated with a higher risk of MRGN colonization (odds ratio [OR], 17.5; 95% CI, 1.6-192). Chronic wounds and gastrostomy were also risk factors of MRGN colonization (OR, 10.7; 95% CI, 1.6-69.3 and OR, 18.3; 95% CI, 2.4-139.4, respectively). There was no difference in mortality between colonized and noncolonized patients.

**Conclusions:** Prevalence of colonization with MRGN bacteria was low in patients in LTCFs and geriatric wards in Graz, Austria.

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Multidrug-resistant gram-negative (MRGN) bacteria are a major public health concern.<sup>1</sup> These organisms are most often discussed in the context of nosocomial infections. However, increasing evidence suggests that residents in long-term care facilities (LTCFs) are an important reservoir of MRGN bacteria.<sup>2</sup> Several studies have demonstrated that residents in LTCFs are colonized by MRGN bacteria with rates varying from 4.7%-64%.<sup>3-6</sup> Asymptomatic carriage of MRGN bacteria harbors the risk of subsequent infection by the multidrug-

resistant organism.<sup>7</sup> In addition, it also constitutes a potential source of transmission to other patients, in particular through hands of health care workers, turning colonization into an important issue in infection control.

In 2012, the Robert Koch Institute (RKI) in Germany issued recommendations on infection control measures dealing with patients infected or colonized by MRGN bacteria, specifically Enterobacteriaceae, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii* isolates.<sup>8</sup> Isolates resistant to 3 out of 4 relevant antimicrobial classes (acylureidopenicillin, third- or fourth-generation cephalosporins, carbapenems, and fluoroquinolones) are in general classified as 3MRGN. Enterobacteriaceae resistant to carbapenems are classified as 4MRGN even if the isolate remains susceptible to 1 other antibiotic class. Isolates resistant to all 4 classes are classified as 4MRGN. The guidance document also includes detailed instructions on hospital infection control measures.<sup>8</sup> This classification is also widely used in Austrian hospitals, including the Medical University Graz Medical Center. Because residents of local LTCFs are often

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Data were presented in part at the 26th European Congress of Clinical Microbiology and Infectious Diseases, Amsterdam, the Netherlands, April 9-12, 2016, and at national conferences during 2016 (Austrian Society of Hygiene, Microbiology, and Preventive Medicine and Austrian Society of Infectious Diseases).

Conflicts of interest: None to report.

transferred to our hospital, we aimed to determine the prevalence of colonization by MRGN bacteria (including Enterobacteriaceae, *P. aeruginosa*, and *A. baumannii*, as defined by RKI<sup>8</sup>) over 6 months in residents of local LTCFs and a geriatric ward and evaluated risk factors for such colonization.

## MATERIALS AND METHODS

### Setting and study design

The Geriatric Health Centre of the City of Graz is a local institution comprising 3 LTCFs (total of 300 beds) and 3 long-term geriatric wards (total of 90 beds) among other facilities. Chronically ill, elderly patients who need constant medical attention by physicians are treated at the geriatric ward. During 2015, the mean length of stay was 147 days in the geriatric wards. In contrast, patients who live in LTCFs do so permanently and only need nursing care and support in their daily activities. LTCFs in Austria generally do not have a permanent physician on call.

We conducted a point-prevalence study in August 2015 that included 137 patients who provided written informed consent: 64 patients from the geriatric wards and 73 residents from LTCFs. Two swabs (Copan, Brescia, Italy) were taken from the perianal region (anus and surrounding skin) and from skin of the inguinal region (pooled from both sides), respectively. Microbiologic sampling was repeated in initially colonized patients 6 months later in February 2016. Health care workers were trained how to obtain microbiologic swabs. Local infection control measures were reviewed with health care workers. Sampling was scheduled during the morning ward round before bathing and dressing the residents. Informed written consent was obtained from the residents or patients individually (henceforth both referred to as patients) or if they were not competent to consent from their legal representatives. The study was approved by the local ethics committee (decision No. 27-378 ex 14/15 Medical University of Graz).

### Data collection

At the initial survey, a structured questionnaire was completed for each participating patient to obtain demographic and administrative data as well as data concerning possible risk factors for asymptomatic colonization by MRGN bacteria. Collected variables included age, gender, length of facility stay, level of care, mobility, bowel and bladder incontinence, dementia or cognitive disorders, previous hospitalization or surgery (in the past 3 months), previous antibiotic use (in the past 3 months), presence of gastrostomy and/or urinary catheters, presence of chronic wounds (decubitus, surgical wounds, or chronic vascular ulcers), or diabetes mellitus. Level of care was defined according to Austrian national standards as levels 1 through 7 (where level 1 = more than 65 hours of care needed per month and level 7 = more than 180 hours of care needed per month and patient unable to move any extremity).<sup>9</sup> Questionnaires for the follow-up survey after 6 months included infections and antibiotic treatment during the past 6 months, as well as cases of death. Follow-up questionnaires were obtained from all patients initially included in the study.

### Microbiologic methods

Microbiologic samples were transported immediately to the microbiology laboratory of the Institute of Hygiene, Microbiology, and Environmental Medicine, Medical University of Graz. Swabs were plated on ChromID ESBL and Chrom ID Carba Smart agars (bioMérieux, Marcy l'Etoile, France). The plates were incubated under aerobic conditions at 36°C and were evaluated for growth after 24

and 48 hours. Suspected colonies were further cultivated on blood agar and identified to species level using the Vitek MS system (bioMérieux). Antimicrobial susceptibility was tested using Vitek-2 (card AST-N196) with interpretation of the results according to European Committee on Antimicrobial Susceptibility Testing breakpoints. Isolates were classified as 3MRGN or 4MRGN according to the RKI guidance document.<sup>8</sup> Extended spectrum  $\beta$ -lactamase (ESBL) production was determined with a Clinical and Laboratory Standards Institute confirmatory test. All isolates were stored at -70°C for analysis of genetic relatedness at the end of the study. Automated repetitive polymerase chain reaction (PCR) with the DiversiLab System (bioMérieux) was performed to determine clonal relationships following the manufacturer's instructions. Isolates with a similarity index >95% were considered related and isolates with a similarity index >97.5% were considered indistinguishable.

### Detection and determination of ESBL genes

If a patient was colonized in the perianal and inguinal region by isolates with the same susceptibility pattern, only 1 isolate was analyzed further. PCR detection and gene identification was performed for the following  $\beta$ -lactamase gene families: *bla*CTX-M-1 group, *bla*CTX-M-2 group, *bla*CTX-M-8 group, *bla*CTX-M-9 group, *bla*GES, *bla*SHV, *bla*TEM, and *bla*VEB. PCR and sequencing procedures were performed as described previously and carried out for all Enterobacteriaceae isolates that showed an ESBL-positive phenotype.<sup>10,11</sup> Total DNA and plasmid DNA isolated with QIAprep Spin Miniprep Kit (Qiagen, Hilden, Germany) were used as templates for PCRs.

### Statistical analysis

Quantitative variables were expressed as mean  $\pm$  standard deviation. For statistical analysis Student *t* test, Cramer *V* test, and Fisher exact test were used as appropriate. Significant variables and variables that showed a trend toward significance ( $P < .1$ ) in the comparison between colonized and noncolonized patients were subsequently included in a binary logistic regression analysis to determine independent risk factors for colonization by MRGN isolates as previously published.<sup>12</sup>  $P < .05$  was considered to indicate statistical significance. The statistical software package SPSS 20.0 (IBM-SPSS Inc, Armonk, NY) was used.

## RESULTS

### Patient characteristics

A total of 137 subjects were included in the study. Demographic and clinical characteristics are summarized in [Appendix 1](#). Patients at the geriatric ward were more often bedridden and experienced bowel and bladder incontinence more often than patients at the LTCFs. A quarter of patients residing in the geriatric ward had received antibiotics during the 3 months before the study compared with 5.6% of LTCF residents ( $P = .001$ ).

### Prevalence of MRGN bacteria

We detected 12 patients harboring 3MRGN isolates (overall prevalence, 8.7%). Five patients were colonized only in the perianal region, 1 patient only in the inguinal region, and 6 patients in both regions. The prevalence of inguinal colonization was 5.1% (7 out of 137), whereas the prevalence of perianal colonization was 8% (11 out of 137). There was no significant difference in the overall prevalence between patients at LTCFs ( $n = 4$  [5.5%]) and the geriatric wards ( $n = 8$  [12.5%]) ( $P = .2$ ). *Escherichia coli* was isolated most frequently (in 9 patients). *Klebsiella pneumoniae* was detected in 2 patients and *P*

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