ORIGINAL ARTICLE EPIDEMIOLOGY

Colonization of residents and staff of a long-term-care facility and adjacent acute-care hospital geriatric unit by multiresistant bacteria

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Abstract

Long-term-care facilities (LTCFs) are reservoirs of resistant bacteria. We undertook a point-prevalence survey and risk factor analysis for specific resistance types among residents and staff of a Bolzano LTCF and among geriatric unit patients in the associated acute-care hospital. Urine samples and rectal, inguinal, oropharyngeal and nasal swabs were plated on chromogenic agar; isolates were typed by pulsed-field gel electrophoresis; resistance genes and links to insertion sequences were sought by PCR; plasmids were analysed by PCR, restriction fragment length polymorphism and incompatibility grouping. Demographic data were collected. Of the LTCF residents, 74.8% were colonized with ≥1 resistant organism, 64% with extended-spectrum β-lactamase (ESBL) producers, 38.7% with methicillin-resistant *Staphylococcus aureus* (MRSA), 6.3% with metallo-β-lactamase (MBL) producers, and 2.7% with vancomycin-resistant enterococci. Corresponding rates for LTCF staff were 27.5%, 14.5%, 14.5%, 1.5% and 0%, respectively. Colonization frequencies for geriatric unit patients were lower than for those in the LTCF. Both clonal spread and plasmid transfer were implicated in the dissemination of MBL producers that harboured IncN plasmids bearing bla_{VIM-1}, *qnrS*, and bla_{SHV-12}. Most (44/45) ESBL-producing *Escherichia coli* isolates had bla_{CTX-M} genes of group 1; a few had bla_{CTX-M} genes of group 9 or bla_{SHV-5}; those with $bla_{CTX-M-15}$ or bla_{SHV-5} were clonal. Risk factors for colonization of LTCF residents with resistant bacteria included age ≥86 years, antibiotic treatment in the previous 3 months, indwelling devices, chronic obstructive pulmonary disease, physical disability, and the particular LTCF unit; those for geriatric unit patients were age and dementia. In conclusion, ESBL-producing and MBL-producing *Enterobacteriaceae* and MRSA were prevalent among the LTCF residents and staff, but less so in the hospital geriatric unit. Education of LTCF employees and better infection control are proposed to minimize the spread of resistant bacteria in the facility.

Keywords: AmpC, extended-spectrum β-lactamases, long-term-care facility, metallo-β-lactamase, MRSA, risk factor analysis, screening, VRE

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Introduction

Resistant bacteria are a major public health concern. Current problems include *Enterobacteriaceae* with extended-spectrum-β-lactamases (ESBLs), derepressed or acquired AmpC cephalosporinases and metallo-β-lactamases (MBLs), and also methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE). These organisms are most often discussed in the context of hospital infection, but are also important in long-term-care facilities (LTCFs), where they have been associated with increased morbidity, mortality and cost [1]. Colonized LTCF residents may act as vectors for the transfer of resistant bacteria into acute-care hospitals, where these may cause infection in their initial host or spread to other vulnerable patients. Donskey found that 15% of hospitalized patients colonized with resistant

Gram-negative bacteria subsequently developed a bacteraemia due to the same strain [2].

Enterobacteriaceae with ESBLs, AmpC enzymes or MBLs are widely seen in clinical samples by the microbiology laboratory in Bolzano, Italy, as are MRSA strains, whereas VRE are rarer. Except for VRE, these organisms are recorded more frequently from LTCF residents than from acute-care hospital patients (R. Aschbacher, unpublished data). Such observations are, however, prone to sampling bias. To test their broader validity, we undertook a point-prevalence study of key resistant pathogens in residents and staff of the LTCF attached to the hospital and in patients of two of the hospital's geriatric units. Particular attention was paid to MBL-producing and ESBL-producing *Enterobacteriaceae*, as these have caused clinical problems in the hospital [3].

Materials and Methods

Facility, patient characteristics, and survey design

In October 2008, we conducted a point-prevalence study concerning MRSA, VRE and of *Enterobacteriaceae* with ESBLs, MBLs or high-level AmpC activity in the 120-bed LTCF attached to the regional hospital in Bolzano, a city of 100 000 people in northern Italy. All residents of all five LTCF units were eligible to participate, and the study was approved by the Ethics Committee of the Bolzano Teaching Hospital. Informed written consent was obtained from the residents or, if they were unable to consent, from their relatives. LTCF staff members were also screened. A parallel 1-day point-prevalence study was performed involving all patients hospitalized in two of the three 24-bed units of the geriatric ward of the acute-care hospital, again with the patients' written consent. The third unit was excluded, as it mostly serves cancer patients with advanced disease. The LTCF and geriatric unit staff move frequently among the various units, but not between the LTCF and the acute-care hospital.

Microbiological methods

To assess carriage of resistant bacteria, midstream or catheter urine samples and rectal, inguinal, oropharyngeal and nasal swabs from all participants were spread on ChromID ESBL, MRSA and VRE agars (bioMérieux, Marcy l'Etoile, France). The formulations of these media are proprietary, but ChromID ESBL is cefpodoxime-based and allows the growth of bacteria with cephalosporin resistance mechanisms other than ESBL production [4]; ChromID MRSA contains cefoxitin (4 mg/L) and ChromID VRE has vancomycin (8 mg/L). The ESBL plates were incubated for 18–24 h at 35°C, whereas the MRSA and VRE plates were incubated for 48 h. Gram-negative bacteria were subcultured on MacConkey agar, and Gram-positive bacteria on colistin–nalidixic acid sheep blood agar. The subcultured isolates were re-identified and tested for antibiotic susceptibility in the hospital's clinical microbiology laboratory, using the Vitek 2 System (bioMérieux), calibrated against CLSI susceptibility criteria [5]. AST-GN015 susceptibility cards, with a direct ESBL test, were used for Gram-negative bacteria, AST-GP049 cards, with both oxacillin and cefoxitin, for MRSA, and AST-GP034 cards for enterococci. Phenotypic identification of β-lactamase types among the isolates growing on ChromID was based on the Vitek 2 results and those of β-lactamase-diagnostic Etests (cefotetan/cefotetan + cloxacillin for AmpC; imipenem/imipenem + EDTA for MBLs; and cefotaxime/cefotaxime + clavulanate, ceftazidime/ceftazidime + clavulanate and cefepime/cefepime + clavulanate for ESBLs; all three were from AB Biodisk, Solna, Sweden). In addition, Hodge/clover leaf plates were used for carbapenem-resistant isolates [6]. VRE were also confirmed by Etest, and equivocal MRSA isolates were tested for penicillin-binding protein 2′, using latex agglutination (Slidex MRSA Detection; bioMérieux). MICs for MBL producers and ESBL-producing *E. coli* isolates were reconfirmed by agar dilution, performed according to BSAC guidelines [7].

Molecular methods

Isolates were typed using pulsed-field gel electrophoresis (PFGE) of *Xba*I-digested genomic DNA [8], with banding patterns analysed using Bionumerics software (Applied Maths, Sint-Martens-Latem, Belgium); they were considered to be clonally related if there was ≥85% similarity in profile [9].

Multiplex PCR for $bla_{\text{CTX-M}}$ genes was performed with published primers [10]. Isolates with MBL phenotypes were tested by PCR with consensus primers for $bla_{\text{VIM-1/2}}$ [11] and $bla_{\text{VIM-1}}$ [12]. Published primers were also used to seek *qnrA* [13], *qnrB* [14] and *qnrS* [15] alleles and bla_{SHV} genes [16]. Sequencing of PCR products derived from bla_{VIM} and *qnrS* was performed with the same primers used for amplification. For bla_{SHV}, primers SHV-c (ATGCGTTATATTCGCCTGTG) and SHV-d (CTTAGCGTTGCCAGTGCTCG) were used, together with other previously described primers [16]. CTX-M group 1 genes were amplified with the primers CTX-M1orfF (PROM+) and CTX-MorfR (PRECTX-M-3-B) [17], and CTX-M group 9 genes with primers CTX-M 9A and CTX-M 9C [18]; sequencing was performed using the same primers together with published internal primers [10]. Published primers were used to amplify the IS*26* insertion element and to investigate linkage of $bla_{\text{CTX-M}}$ genes of group 1 with IS*Ecp1* and IS*26* [19]. In all cases, products were first

purified with the Geneclean Turbo for PCR Kit (Q-BIOgene, Cambridge, UK), with sequencing by the GenomeLab Dye terminator Cycle Sequencing system, using the Quick Start Kit (Beckman Coulter, High Wycombe, UK) with a Beckman Coulter CEQ 8000 Genetic Analysis System.

Mega X *E. coli* DH10B T1 electrocompetent cells (Invitrogen, Paisley, UK) were transformed by electroporation with a Bio-Rad Gene-Pulser II at 2.0 kV, 200 Ω and 25 μF, using plasmids extracted by the method of Kado and Liu, and precipitated twice with ethanol [20]. Transformants were selected on Luria–Bertani agar containing cefotaxime (2 mg/L). Plasmids bearing bla_{VIM-1} were extracted from transformants and digested with *Hpa*I (Promega, Southampton, UK) and *Bam*HI/*Sac*I (Roche, Mannheim, Germany). The resulting fragments were separated by electrophoresis on 1% agarose.

Plasmid typing was by PCR (inc/rep PCR) for the major incompatibility groups [21]. Phylogenetic groups of *E. coli* were determined by PCR [22].

Epidemiological investigation and statistical analysis

To examine risk factors for colonization with resistant organisms, in-house physicians reviewed hospital records and, using an Epi Info questionnaire, recorded demographic data as follows: patient age, gender, length of stay in the LTCF or geriatric ward, diagnosis at admission, Barthel immobility score, comorbidities (dementia, urinary incontinence, diabetes, cancer, vascular diseases, chronic obstructive pulmonary disease, decubitus ulcers and immunosuppressive therapy), and indwelling medical devices (urinary catheters, percutaneous enteral gastrostomy tubes, tracheostomy tubes and nasogastric tubes). For the LTCF patients, the hospital ward of the previous acute admission, antibiotic treatments in the preceding 3 months and other possible risk factors (urinary incontinence, decubitus ulcers and immunosuppressive therapy) were also recorded. For statistical analysis, three groups of subjects were defined: (i) LTCF residents; (ii) LTCF staff; and (iii) geriatric unit patients. Associations were investigated using the chi-square test or Fisher's exact method. Group-specific mean ages were compared by the generalized linear model procedure, after testing for homoschedasticity of variances with Levene's test. In the case of heteroschedasticity, Welch's test was performed. Logistic regression analyses were developed to investigate colonization with resistant bacteria in general and ESBL producers and MRSA in particular, first as univariate and then as multivariate models through stepwise selection. Analyses were performed using the SAS statistical package, release 9.1.3, and with Epi Info v. 3.5.

Results

Epidemiological investigation

Among 120 LTCF residents present in October 2008, 111 (92.5%) agreed to participate in the point-prevalence survey; among staff members, 69 of 79 (87.3%) agreed to participate, mainly nurses and physicians. Sixty-one (55.0%) of the participating residents were women, with a median age of 84 years (range 22–96 years); the median age of the 50 male residents was 77 years (range 22–94 years). The median length of LTCF stay was 21 months (range: <1–96 months). The most recent hospital admission of 36 of the 111 participating LTCF residents was to one of the two acute-care geriatric units that were also surveyed. Forty-five of the 48 geriatric unit patients also agreed to participate in the investigation: 32 (71.1%) were women (median age 85 years, range 75–97 years) and 13 were men (median age 79 years, range 74–91 years); none was ordinarily a resident in the LTCF.

The isolation frequencies for the various resistance types in different patient groups are shown in Table 1. Among the LTCF residents, 74.8% (83/111) were colonized with at least one resistant organism, and 31.5% (35/111) with at least two. ESBL-producing *Enterobacteriaceae*, together with MRSA strains, were isolated from 28.8% (32/111), and at least two different ESBL-producing enterobacterial species from 21.6% (24/111). Twenty-seven of 111 LTCF residents, two of 69 LTCF staff members and two of 45 geriatric unit patients were also colonized with *Acinetobacter baumannii* (which grew on the ESBL ChromID agar), but none of these isolates was resistant to carbapenems, and they were not studied further. Similarly, colonization with *Pseudomonas aeruginosa* was not investigated.

ESBL-producing *Enterobacteriaceae* and, more surprisingly, MRSA isolates were most often recovered from rectal samples. The best combinations of screening measures to recognize patients colonized with key resistant bacteria were a rectal swab and a urine sample for ESBL producers, and both inguinal and oropharyngeal swabs for MRSA (Table 2). Testing only nasal swabs would have resulted in substantial underestimation of colonization with MRSA.

MICs for, and molecular typing of, MBL-producing strains

Ten MBL-producing isolates were found; eight from LTCF residents (one with two different producers), one from a staff member, and one from a geriatric unit patient (Table 3). All had bla_{VIM-1} together with *qnrS*. The PFGE patterns of two bla_{VIM}-positive *Klebsiella oxytoca* isolates from LTCF residents were identical, whereas the patterns of the two MBL-produc-

TABLE 1. Long-term-care facility (LTCF) residents, staff and geriatric unit patients colonized with bacteria of various resistance types

Bacteria and resistance type	Screening samples; % colonized			
	LTCF (residents), *n* = 111	LTCF (staff), *n* = 69	Geriatrics unit (patients), *n* = 45	LTCF residents vs. geriatrics unit patients: p-value of differences
All resistance groups	74.8	27.5	22.2	<0.0001
All enterobacteria, ESBL-positive[a]	64.0	14.5	8.9	<0.0001
Escherichia coli, ESBL-positive	41.4	11.6	6.7	<0.0001
Proteus mirabilis, ESBL-positive	24.3	1.5	2.2	<0.0001
Klebsiella pneumoniae, ESBL-positive	11.7	1.5		0.034
Morganella morganii, ESBL-positive	7.2		2.2	0.45
Citrobacter koseri, ESBL-positive	3.6			
Klebsiella oxytoca, ESBL-positive	1.8			
Enterobacter cloacae, ESBL-positive	0.9			
Serratia fonticola, ESBL-positive		1.5		
All enterobacteria, MBL-positive	5.4[b]	1.5	2.2	0.67
E. coli, MBL-positive	1.8	1.5		
K. pneumoniae, MBL-positive	1.8			
K. oxytoca, MBL-positive	1.8			
Citrobacter freundii, MBL-positive			2.2	
All enterobacteria, high-level AmpC	4.5	1.5	6.7	0.69
E. cloacae, high-level AmpC	3.6		2.2	1.0
E. coli, high-level AmpC	0.9		2.2	0.49
Serratia marcescens, high-level AmpC			2.2	
Hafnia alvei, high-level AmpC		1.5		
MRSA	38.7	14.5	6.7	<0.0001
VRE[c]	2.7			0.56

ESBL, extended-spectrum β-lactamase; MRSA, methicillin-resistant *Staphylococcus aureus*; VRE, vancomycin-resistant enterococci.
[a]Enterobacteria producing both an ESBL and an MBL are included in both totals.
[b]Urine sample of one further patient tested positive for two MBL-producing enterobacterial species (*Providencia stuartii*, *M. morganii*) shortly before the screening period; inclusion of these samples brings the MBL-positive rate to 6.3% (7/111).
[c]All three VRE were *Enterococcus faecalis*.

TABLE 2. Percentages of patients, residents or staff found to be positive for methicillin-resistant *Staphylococcus aureus* (MRSA) or extended-spectrum β-lactamase (ESBL) producers with various specimen type combinations

	MRSA (%)		ESBL producers (%)	
	All study populations pooled	LTCF residents	All study populations pooled	LTCF residents
Rectal	61[a]	69	96	95
Inguinal	59	67	73	82
Oropharyngeal	53	50	35	40
Nasal	48	45		
Urine			23	43
Rectal + inguinal	75	86	97	97
Rectal + oropharyngeal	77	81	96	95
Rectal + nasal	80	81		
Rectal + urine			99	98
Inguinal + oropharyngeal	82	88	73	82
Inguinal + nasal	80	81		
Inguinal + urine			80	91
Oropharyngeal + nasal	77	69		
Oropharyngeal + urine			60	68

LTCF, long-term-care facility.
[a]Proportion of all positives with the group detected by indicated sample type.

ing *E. coli* isolates from LTCF residents and the one from a staff member were >80% related. All three *E. coli* isolates belonged to phylogenetic group B2. MICs of imipenem for the MBL producers ranged from 2 to 16 mg/L, and were reduced to ≤0.5 mg/L in the presence of 320 mg/L EDTA, except in the case of *Morganella morganii* and *Providencia stuartii* isolates—species that are inherently less susceptible to imipenem. MICs of meropenem and ertapenem for the MBL producers generally were lower than those of imipenem, but above those for MBL-negative isolates. MICs of ciprofloxacin ranged from 1 to >8 mg/L. Two MBL producers were highly susceptible to aztreonam, with MICs ≤0.25 mg/L, whereas the aztreonam MIC for the *P. stuartii* isolate was 4 mg/L; values were ≥64 mg/L for the other seven isolates. Five of the seven aztreonam-resistant MBL producers, excluding only the two *Klebsiella pneumoniae* isolates, were examined by

TABLE 3. MICs (mg/L) for the metallo-β-lactamase-producing *Enterobacteriaceae*, *Escherichia coli* DH10B, and a representative transformant

Antibiotic	Geriatric unit	LTCF staff	LTCF residents							Recipient	Transformant
	Citrobacter freundii, 208	*Escherichia coli*, 165	*E. coli*, 6	*E. coli*, 95	*Morganella morganii*, 445	*Providencia stuartii*, 446	*Klebsiella pneumoniae*, 20	*K. pneumoniae*, 57	*Klebsiella oxytoca*, 41	*K. oxytoca*, 105	*E. coli* DH10B	DH10B (pKOX105)
Ampicillin	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	8	>64
Amoxicillin–clavulanate 2 : 1	32	32	32	64	>64	>64	32	32	32	32	8	64
Aztreonam	0.25	≤0.125	64	>64	>64	4	>64	>64	>64	>64	≤0.125	>64
Cefotaxime	128	128	128	>256	256	8	>256	64	256	128	≤0.125	64
+4 mg/L clavulanate	>32	>32	>32	16	32	4	>32	16	>32	>32	≤0.060	>32
Ceftazidime	>256	256	256	256	64	16	>256	>256	>256	>256	0.5	256
+4 mg/L clavulanate	>32	>32	>32	>32	32	4	>32	>32	>64	>32	0.25	>32
Cefpirome	>64	64	>64	>64	64	4	>64	32	>64	64	≤0.125	32
+4 mg/L clavulanate	>32	>32	>32	16	8	8	32	16	>32	>32	≤0.060	32
Cefoxitin	>64	>64	>64	64	>64	4	64	32	>64	>64	8	64
Piperacillin–tazobactam	>64	>64	>64	64	4	16	>64	64	>64	>64	4	>64
Imipenem	8	8	8	4	16	2	4	4	8	4	0.5	8
+400 mg/L EDTA	0.5	0.125	0.125	0.5	4	2	0.25	0.125	0.25	0.25	0.25	0.25
Meropenem	2	2	2	0.5	0.5	≤0.060	1	0.5	4	4	≤0.060	1
Ertapenem	1	1	1	0.5	0.25	≤0.125	1	1	4	2	≤0.125	0.5
Ciprofloxacin	>8	>8	>8	>8	4	>8	1	1	8	8	≤0.125	0.5
Tobramycin	16	16	16	16	16	8	4	4	16	16	0.5	4
Gentamicin	>32	>32	2	2	>32	4	1	2	4	2	0.5	1
Amikacin	2	2	4	8	4	1	2	2	2	4	1	1
Minocycline	>32	2	1	2	>32	32	2	4	1	1	1	0.25
Tigecycline	1	≤0.25	≤0.25	≤0.25	2	2	0.5	0.5	≤0.25	≤0.25	≤0.25	≤0.25
Colistin	1	≤0.5	≤0.5	1	>32	>32	16	16	1	1	≤0.5	≤0.5

LTCF, long-term-care facility.

PCR and found to be positive for bla_{SHV}. One bla_{VIM-1}-positive *E. coli* isolate (aztreonam MIC >64 mg/L) also had a bla_{CTX-M} gene belonging to group 1. Except for the *P. stuartii* isolate, the MBL-positive isolates had high-level resistance to oxyimino-cephalosporins (≥32 mg/L) and cefoxitin (≥32 mg/L), with weak synergy, at most, with clavulanate, generally reducing the cephalosporin MIC ≤4-fold.

Three MBL producers from LTCF residents (*E. coli* 6, *K. oxytoca* 105, and *M. morganii* 445) were used as plasmid donors for successful transformation of *E. coli* DH10B. All of the transformants were PCR-positive for bla_{VIM-1}, *qnrS*, and bla_{SHV}, and contained plasmids designated pECO6, pKOX105.1, and pMMO445, respectively. We also obtained a transformant with a derivative plasmid pKOX105.2 from *K. oxytoca* 105; this carried bla_{VIM-1} but lacked *qnrS* and bla_{SHV}. The transferred plasmids pECO6 and pKOX105.1 (Fig. 1, lanes 1 and 2) were identical by digestion with *Hpa*I (and *Bam*HI/*Sac*I; data not shown) and very similar to pKOX105.2 and pMMO445 (Fig. 1, lanes 3 and 4). All of the plasmids belonged to incompatibility group IncN.

MICs of, and molecular typing of, ESBL-producing *E. coli* isolates

Forty-two of 46 ESBL-producing *E. coli* isolates from LTCF residents, all eight from staff members and all three from the geriatric unit patients were tested further. In all cases, MICs were ≥2 mg/L for at least one of cefotaxime, ceftazidime or cefpirome (Fig. 2), and were reduced eight-fold or more in the presence of clavulanate (4 mg/L) whereas cloxacillin

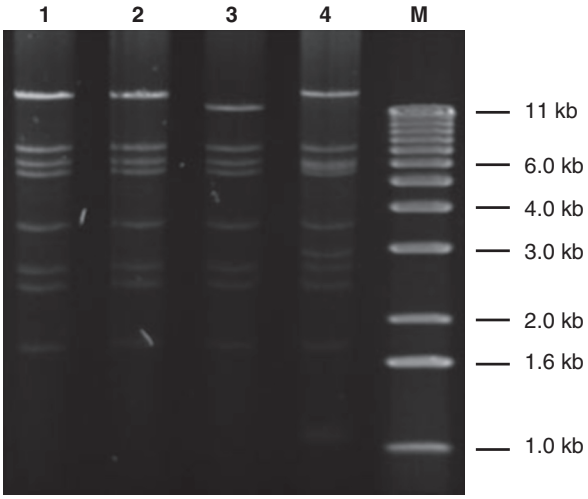

FIG. 1. Plasmids extracted from *Escherichia coli* DH10B transformants, digested with *Hpa*I and separated on a 0.7% agarose gel: pECO6 (lane 1), pKOX105.1 (lane 2), pKOX105.2 (lane 3) and pMMO445 (lane 4) were from isolates collected in the surveillance of long-term-care facility residents. Lane M has molecular weight markers.

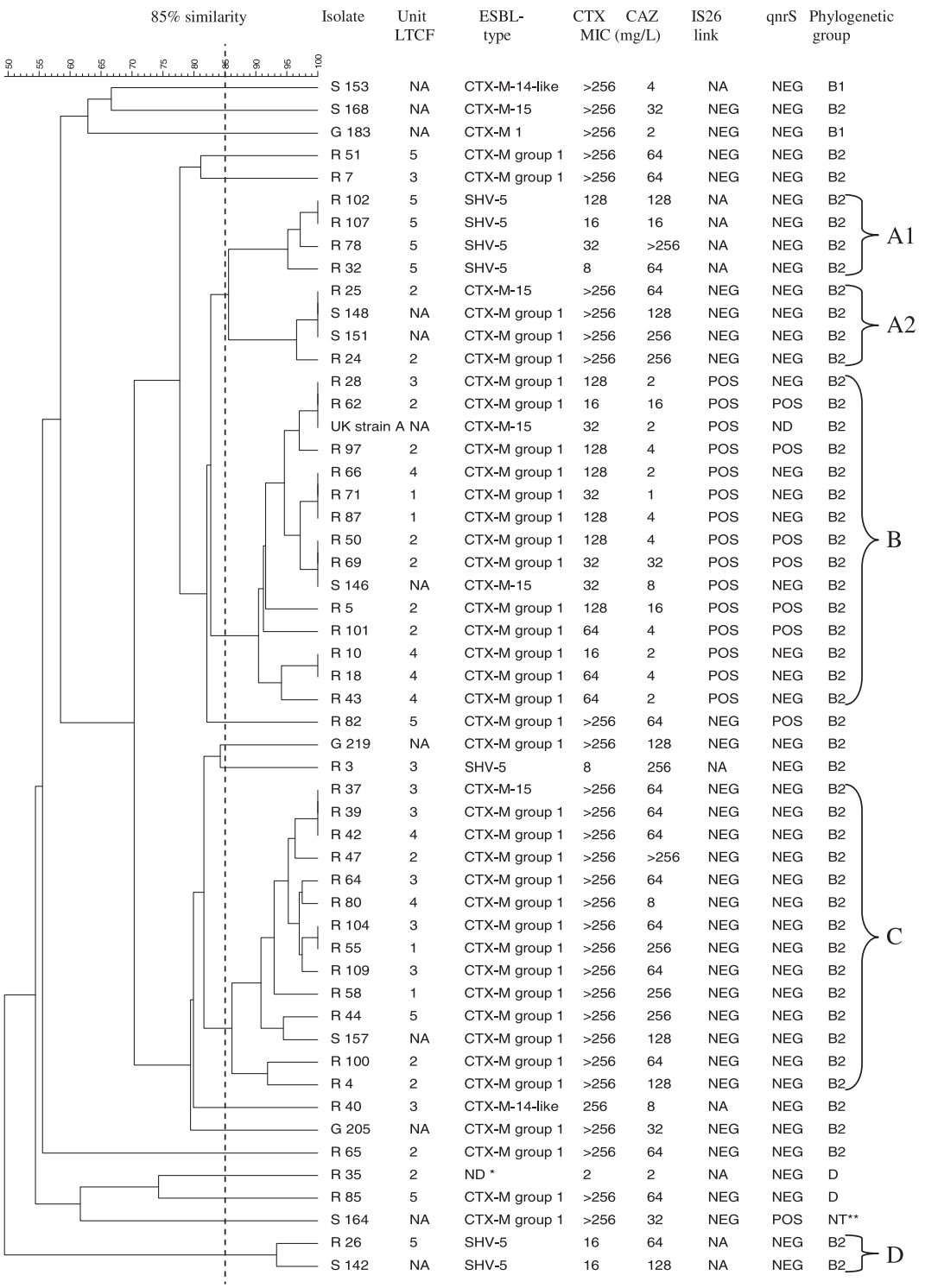

FIG. 2. Pulsed-field gel electrophoresis (PFGE) dendrogram of the extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* isolates from the screening of the long-term-care facility (LTCF) residents (R) and staff members (S) and geriatric unit patients (G). Specific LTCF unit, ESBL type, cefotaxime (CTX) and ceftazidime (CAZ) MICs, the presence of IS*26* adjacent to $bla_{\text{CTX-M-group-1}}$ ('IS*26* link'), presence of *qnrS* and phylogenetic group are shown as A1, A2, B, C or D. PFGE clusters with >85% similarity are indicated. NA, not applicable; ND*, ESBL type not determined (SHV-negative and CTX-M negative); NT**, not typeable by the PCR method.

(100 mg/L) had no significant effect. All were resistant to ampicillin and piperacillin, whereas amoxicillin–clavulanate (2 : 1) MICs varied between 8 and 32 mg/L. All but three (*E. coli* R35, S168 and S183 with MICs ≤0.125 mg/L) were substantially resistant to ciprofloxacin, with MICs ≥8 mg/L, whereas the MICs of aminoglycosides were bimodally distributed, with 23 of 53 isolates being susceptible to tobramycin (4 mg/L) and 28 of 53 being resistant at ≥32 mg/L; 37 of 53 were susceptible to gentamicin (4 mg/L) and 15 of 53 were resistant at ≥32 mg/L and 39 of 53 were susceptible to amikacin (8 mg/L), but 14 of 53 were resistant at ≥16 mg/L. All remained susceptible to imipenem (0.5 mg/L), meropenem (0.06 mg/L), ertapenem (1 mg/L), tigecycline (1 mg/L) and colistin (1 mg/L).

PFGE defined clusters A1, A2, B, C and D among 52 ESBL-producing *E. coli* isolates (one isolate was not typeable). Cluster A1 contained four bla_{SHV-5}-positive isolates, whereas two further isolates with this enzyme formed cluster D. All but one of these bla_{SHV-5}-positive isolates were from residents of LTCF unit 5; the exception was from a staff member. Clusters A2 and C contained 18 isolates with bla_{CTX-M} genes of group 1 (one gene from each cluster was sequenced and identified as $bla_{CTX-M-15}$), all with cefotaxime MICs >256 mg/L and ceftazidime MICs generally ≥64 mg/L. Another 14 isolates with bla_{CTX-M} genes of group 1(one identified by sequencing as $bla_{CTX-M-15}$) formed cluster B: cefotaxime and ceftazidime MICs for these were ≤128 mg/L and ≤32 mg/L, respectively, and PFGE patterns closely resembled that of UK strain A [19]. Two unique isolates had CTX-M-14-like enzymes, on the basis of partial sequencing and distinct PFGE patterns, whereas one, also with a unique PFGE pattern, had $bla_{CTX-M-1}$ with insertion of IS*Ecp1* 80 bp upstream. One ESBL-positive isolate had only bla_{TEM}: cefotaxime and ceftazidime MICs for this isolate were 2 mg/L, decreasing to ≤0.25 mg/L in the presence of clavulanate; we did not seek to identify its enzyme.

PCR for the linkage of bla_{CTX-M} genes of group 1 with upstream IS*Ecp1* gave an approximately 600-bp fragment for isolates in clusters A2 and C, and sequencing revealed an IS*Ecp1*-like element 48 bp upstream of the β-lactamase start codon. A slightly larger fragment was seen in strain G183, which had $bla_{CTX-M-1}$, whereas all 14 isolates belonging to cluster B had IS*26* upstream of bla_{CTX-M} genes of group 1, as in UK strain A [19]. Only four of the cluster B isolates gave amplification products in the linkage assay for IS*Ecp1* and bla_{CTX-M} genes of group 1, all with a fragment length compatible with insertion of IS*26* in the inverted repeat sequence of IS*Ecp1*, as in UK strain A [19]; the other ten cluster B isolates did not give a PCR product. Eight ESBL-positive bla_{VIM}-negative isolates had *qnrS*; six of these belonged to cluster B, and none had *qnrA* or *qnrB*.

Risk factors for colonization

Colonization of LTCF residents with resistant organisms, including ESBL producers and MRSA, was associated with several risk factors in univariate and multivariate analysis (Table 4). Age >86 years was an independent risk factor for ESBL producers among LTCF residents, as was administration of antibiotics within the previous 3 months. An association with previous antimicrobial treatment was marginally significant for MRSA (OR 2.31; 95% CI 1.00–5.34; p 0.050); prior treatment with fluoroquinolones was specifically associated with MRSA colonization in univariate analysis only. Univariate analysis also revealed that the presence of any invasive medical device was a significant risk factor for colonization with resistant bacteria, ESBL producers or MRSA, and indicated an association between colonization with ESBL producers and the presence of a percutaneous enteral gastrostomy tube, tracheostomy tube or indwelling urinary catheter, and also between colonization with resistant bacteria and a urinary catheter. MRSA colonization was weakly associated with a nasogastric tube (OR 8.82, 95% CI 0.99–78.3, p 0.051). Chronic obstructive pulmonary disease was an independent risk factor for MRSA, but the most significant risk factor for MRSA, ESBL producers and all resistant bacteria in LTCF residents was chronic immobility, based on a Barthel index score of 0.

Being resident in unit 1 of the LTCF was associated with significantly less colonization by all resistant bacteria, ESBL producers and MRSA; residence in unit 3 was associated with significantly lower carriage of ESBL producers and MRSA than residence in the other three LTCF units. Of the eight MBL-producing *Enterobacteriaceae* from LTCF residents, five (from four residents) were from unit 4, two were from unit 2 and one was from unit 5, whereas four of the five *Enterobacteriaceae* with high-level AmpC production were isolated from residents in unit 4 and one was isolated from a resident in unit 5. The three VRE—all *Enterococcus faecalis* with the VanA phenotype—were from patients in units 3, 4 and 5. Dementia without chronic immobility was associated with a reduced risk for colonization with ESBL producers and all resistant bacteria in the univariate but not in the multivariate analysis, and this may explain the lower resistance rates in LTCF unit 1, which cares for residents with the behavioural and psychological symptoms of dementia (agitation/aggression, delusions, anxiety, aberrant motor behaviour, hallucinations, etc.).

Age ≥86 years was also a risk factor for colonization with all resistant bacteria in the geriatric units (Table 5); here, however (and in contrast to the LTCF), dementia was a positive risk factor for colonization with all resistant bacteria.

TABLE 4. Univariate and multivariate logistic regression analysis of risk factors for colonization of long-term-care facility (LTCF) residents with resistant bacteria

		Colonization with MRSA				Colonization with ESBL producers				Colonization with resistant bacteria[a]			
		Univariate analysis		Multivariate analysis		Univariate analysis		Multivariate analysis		Univariate analysis		Multivariate analysis	
Variable	111 Residents (%)	OR (95% CI)	p-Value	OR (95% CI)	p-Value	OR (95% CI)	p-Value	OR (95% CI)	p-Value	OR (95% CI)	p-Value	OR (95% CI)	p-Value
Male sex	45.0	0.95 (0.44–2.04)	0.88			1.18 (0.54–2.57)	0.68			0.93 (0.39–2.19)	0.86		
Age ≥86 years	32.4	0.67 (0.25–1.82)	0.44			1.6 (0.57–4.49)	0.37	4.63 (1.12–19.1)	0.034	0.84 (0.29–2.41)	0.74		
Antibiotics in last 3 months	28.8	2.31 (1.00–5.34)	0.05			8.25 (2.40–30.3)	0.0009	7.51 (1.48–38.0)	0.015	ND	ND		
Fluoroquinolones	28.1	6.42 (1.27–32.5)	0.025	5.81 (0.84–40.3)	0.075	4.95 (0.60–41.1)	0.14			ND	ND		
Penicillins	59.4	1.18 0.43–3.23)	0.74			3.59 (0.98–13.2)	0.054			ND	ND		
Cephalosporins	6.3	1.6 (0.10–26.2)	0.74			ND	ND			ND	ND		
Dementia	66.7	0.64 (0.29–1.42)	0.27			0.29 (0.11–0.74)	0.01			0.25 (0.08–0.80)	0.018		
Peripheral vascular disease	75.7	1.7 (0.67–4.31)	0.27			1.6 (0.66–3.87)	0.29			2.16 (0.84–5.52)	0.11		
Incontinence	96.4	ND	ND			ND	ND			ND	ND		
Diabetes	27	1.07 (0.46–2.53)	0.87			0.65 (0.28–1.54)	0.33			0.72 (0.28–1.82)	0.48		
Cancer	19.8	1.41 (0.55–3.63)	0.47			0.77 (0.30–2.01)	0.59	0.22 (0.04–1.07)	0.059	0.66 (0.24–1.84)	0.43		
Decubitus ulcer	9.9	1.36 (0.39–4.76)	0.63			1.57 (0.39–6.27)	0.52			1.58 (0.32–7.79)	0.57		
Chronic obstructive pulmonary disease	6.3	10.8 (1.26–93.7)	0.03	11.7 (1.13–120.8)	0.039	3.6 (0.42–31.0)	0.24			ND	ND		
Physical disability (Barthel score of 0)	74.8	4.06 (1.28–12.9)	0.017			16.7 (5.13–54.7)	<0.0001	11.4 (2.34–55.5)	0.003	23.1 (7.43–71.8)	<0.0001	19.2 (5.82–63.6)	<0.0001
Any medical device	48.6	3.09 (1.40–6.84)	0.005	2.26 (0.87–5.85)	0.093	6.14 (2.47–15.2)	<0.0001			7.83 (2.50–4.6)	0.0004	4.59 (1.24–16.9)	0.022
Percutaneous enteral gastrostomy tube	27	1.57 (0.67–3.67)	0.29			12.3 (2.76–55.4)	0.001	3.2 (0.99–10.37)	0.053	ND	ND		
Tracheostomy tube	18	1.07 (0.40–2.87)	0.89			14.2 (1.83–111)	0.011			ND	ND		
Urinary catheter	31.5	2.16 (0.95–4.89)	0.065			3.91 (1.46–10.5)	0.007			3.58 (1.14–11.3)	0.029		
Nasogastric tube	5.4	8.82 (0.99–78.3)	0.051			ND	ND			ND	ND		
LTCF unit 1	20.8	0.05 (0.01–0.39)	0.004			0.13 (0.04–0.36)	<0.0001			0.07 (0.02–0.20)	<0.0001		
LTCF unit 2	21.6	1.45 (0.58–3.63)	0.42	12.4 (1.26–122)	0.031	8.53 (1.89–38.5)	0.005	7.63 (0.91–64.0)	0.061	4.69 (1.03–21.4)	0.046		
LTCF unit 3	21.6	2.8 (1.11–7.07)	0.029	30.9 (3.18–301)	0.003	0.38 (0.15–0.96)	0.04			1.02 (0.36–2.88)	0.97		
LTCF unit 4	18.9	0.97 (0.36–2.57)	0.94	8.17 (0.79–84.2)	0.077	2.04 (0.69–6.06)	0.2			4.6 (1.46–14.3)	0.009		

ESBL, extended-spectrum β-lactamase; MRSA, methicillin-resistant *Staphylococcus aureus*; ND, not defined.
[a]Colonization with at least one of the following: MRSA, vancomycin-resistant enterococci, *Enterobacteriaceae* with ESBLs, metallo-β-lactamases, or derepressed or acquired AmpC.

TABLE 5. Univariate and multivariate logistic regression analysis of risk factors associated with colonization of geriatric unit patients with resistant bacteria

Variable	45 Patients (%)	Colonization with resistant bacteria of any or of the types studied[a]			
		Univariate analysis		Multivariate analysis	
		OR (95% CI)	p-value	OR (95% CI)	p-value
Male sex	28.9	0.55 (0.10–3.00)	0.48		
Age ≥86 years	37.8	3.27 (0.78–13.9)	0.11	6.84 (1.11–42.3)	0.039
Antibiotics in last 3 months	33.3	2.5 (0.59–10.5)	0.21	5.68 (0.62–34.9)	0.061
Fluoroquinolones	26.7	1.19 (0.11–12.8)	0.89		
Penicillins	46.7	1.5 (0.24–9.22)	0.66		
Cephalosporins	26.7	1.19 (0.11–12.8)	0.89		
Dementia	33.3	4.33 (0.99–18.9)	0.051	9.52 (1.47–61.8)	0.018
Peripheral vascular disease	80	2.67 (0.29–24.3)	0.38		
Incontinence	57.8	1.97 (0.44–8.87)	0.38		
Diabetes	24.4	0.28 (0.03–2.49)	0.25		
Cancer	24.4	0.72 (0.13–4.05)	0.71		
Decubitus ulcer	8.9	4.13 (0.50–33.9)	0.19		
Chronic obstructive pulmonary disease	17.8	0.44 (0.05–4.12)	0.47		
Any medical device	22.2	1.67 (0.39–7.19)	0.49		
Urinary catheter	28.9	1.07 (0.23–4.99)	0.93		
Nasogastric tube	2.2	ND	ND		

ND, not defined.
[a]See listing in Table 4.

Discussion

We evaluated colonization with resistant bacteria among residents of a LTCF and two linked geriatric units of the acute-care hospital in Bolzano. Overall, 74.8% of LTCF residents were colonized with at least one target organism, most frequently an ESBL producer (64.0%), MRSA (38.7%), MBL producer (6.3%) or AmpC hyperproducer (4.5%). VRE colonization was rare (2.7%). Many residents had more than one target organism, underscoring the role of LTCFs as a reservoir for these organisms [1,23,24]. MRSA colonization among LTCF residents has long been recognized, sometimes with colonization frequencies ≥50% [1,24,25].

Carriage of *Enterobacteriaceae* with VIM MBLs by 6.3% of LTCF residents is of special concern: MBL-producing *Enterobacteriaceae* are rare in Europe, except for Greece [26], where plasmids encoding the VIM-1 enzyme have spread among *K. pneumoniae* strains [27]. An Italian countrywide survey in 2004 identified only one MBL producer (an *Enterobacter cloacae* isolate) among over 12 000 *Enterobacteriaceae* screened [28], with further recent single isolates [29] or clusters being found in Genoa [30] and Bolzano [3]. The present data imply: (i) resident-to-resident transfer of MBL producers in the LTCF, on the basis of identical or similar PFGE patterns for a pair of *K. oxytoca* isolates and for three *E. coli* isolates, one of them from a staff member; and (ii) strain-to-strain plasmid transfer, on the basis of identical or related IncN plasmids with bla_{VIM-1} and *qnrS* in multiple *Enterobacteriaceae* species. The plasmids from the LTCF residents' isolates had very similar restriction patterns to clinical strains collected up to 3 years previously [3].

Many colonizing *Enterobacteriaceae* had ESBL genes: principally, bla_{CTX-M} genes of group 1 (81%), $bla_{CTX-M-14}$ (4%) and bla_{SHV-5} (13%). The host isolates of these genes included four major clusters as determined by PFGE, designated A1, A2, B and C. Cluster B isolates resembled the UK strain A, an ST131 variant, in: (i) PFGE profile (Fig. 2); (ii) having an IS*26* element upstream of $bla_{CTX-M-15}$; and (iii) antibiogram, with lower-level cephalosporin resistance, probably contingent on the separation of $bla_{CTX-M-15}$ from its usual promoter in IS*Ecp1* [19]. Strain A is widespread in the UK and was previously identified from a patient in Austria who had visited southern Italy but not the UK [31]. All three MBL-producing *E. coli* isolates and 90% of ESBL-producing *E. coli* isolates belonged to phylogenetic group B2, whereas 3.8% of the ESBL producers belonged to group D. These phylogenetic groups—particularly B2—account for most virulent extra-intestinal strains of the species [22].

LTCF outbreaks of *E. coli* with CTX-M-15 (the commonest group 1 type) were reported in 2000–2002 in Canada [32], and a survey in northern Italy in 2006–2007 revealed them in 9.1–100% of urine samples from LTCF residents with indwelling catheters [33].

Movement of patients and staff between the LTCFs and hospitals may facilitate dissemination of resistant bacteria. It is unclear whether most *de novo* acquisition occurs in the LTCF or during occasional hospitalizations [1], but the lower colonization rates among the geriatric unit patients argue against the hospital as the main source, as does the fact that, during

2008, the prevalence of ESBL producers among routine clinical isolates from LTCF residents far exceeded that among geriatric unit patients (40% vs. 9%; p <0.0001). A striking feature was the colonization of 27.5% of LTCF staff with resistant bacteria: 14.5% with ESBL producers, 14.5% with MRSA, and one nurse with a bla_{VIM-1}-positive *E. coli* strain. This carriage probably reflects resident-to-staff and, perhaps, staff-to-staff transmission. Elsewhere, MRSA colonization rates of LTCF staff of 7.5–22.7% [34,35] have been reported.

Risk factors found here for colonization with ESBL producers in LTCF residents included treatment with antibiotics within the preceding 3 months, whereas invasive medical devices were associated with both ESBL producers and MRSA. These findings support published data: prior antibiotic treatment is a well-recognized risk factor for colonization with resistant organisms [25], including ESBL producers [1] and MRSA [24], whereas invasive medical devices are considered to be important for ESBL producers [36] and MRSA [24,25]; chronic obstructive pulmonary disease was found to be an independent risk factor for MRSA colonization among LTCF residents in our study and by others [37]. However, the most important risk factor for carriage of resistant organisms, ESBL producers and MRSA was the particular LTCF unit of residence. This is explicable, because the five units manage residents with different levels of independence, basal disease, comorbidity and functional status, all of which influence the frequency and nature of staff contact. Residents in LTCF units 2 and 5 are non-ambulatory and require extensive assistance with daily living activities, along with nursing and medical care; those in units 3 and 4 have less functional disability or comorbidity; and those in unit 1 have dementia but are ambulatory. The large number of ambulant, and relatively autonomous, residents in unit 1 may explain the inverse association between dementia and resistant bacteria found here. A converse, positive, association between resistant bacteria and dementia in the geriatric units possibly indicates acute events leading to hospitalization, along with poor adherence of these patients to hygienic measures as compared with non-dementia patients.

To conclude, we found frequent colonization with resistant organisms among LTCF residents and staff, and much lower rates in acute-care geriatric units. This difference can probably be explained by different risk factors. Patients transferred from the LTCF to acute-care hospitals should be considered to be at high risk of carrying resistant organisms and should be screened on admission. Screening should include a rectal swab and a urine sample for ESBL and MBL producers, and inguinal and oropharyngeal (not just nasal) swabs for MRSA. Because LTCFs are also homes, hospital infection control guidelines are unrealistic, but education of the employees, improvement of hand hygiene, necessary use of gloves and gowns, monitoring of diseases and treatment are all keys to better management, as is ongoing surveillance.

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Transparency Declaration

The microbiological work in Bolzano was partly financed by research grant number 2129 of the Bolzano Hospital. D. M. Livermore has shareholdings, or acts as enduring attorney for a shareholder, in AstraZeneca, Dechra, EcoAnimal Health, GlaxoSmithKline, Pfizer and Schering-Plough; he has had research contracts, or conference finance, in the past 3 years from AstraZeneca, Calixa Cerexa, Johnson & Johnson, Merck, Novartis, Novexel, Pfizer, Phico, Theravance and Wyeth. He is employed by the Health Protection Agency, and is also influenced by their views on antibiotic usage. Other authors have no conflicts of interest to declare.

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