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FULL PAPER

Colonization of residents and staff of an Italian long-term care facility and an adjacent acute care hospital geriatric unit by multidrug-resistant bacteria

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SUMMARY

In 2016, we undertook a point prevalence screening study for *Enterobacteriaceae* with extended-spectrum β-lactamases (ESBLs), high-level AmpC cephalosporinases and carbapenemases, and also methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin resistant enterococci (VRE) in a long-term care facility (LTCF) and the associated acute care hospital geriatric unit in Bolzano, Northern Italy.
Urine samples and rectal, inguinal, oropharyngeal and nasal swabs were plated on selective agars. Demographic data were collected. ESBL and carbapenemase genes were sought by PCR.
We found the following colonization percentages with multidrug-resistant (MDR) bacteria in 2016 in LTCF residents: all MDR organisms, 66.1%; ESBL producers, 53.0%; carbapenemase-producers, 1.7%; MRSA, 14.8%; VRE, 0.8%. Colonization by all MDR bacteria was 19.4% for LTCF staff and 26.0% for geriatric unit patients. PCR showed that 80.3% of *Escherichia coli* isolates from LTCF residents, all *E. coli* isolates from LTCF staff, 62.5% and 100% of *Klebsiella pneumoniae* from LTCF residents and geriatric unit patients, respectively, had a bla_{CTX-M}-type gene. All carbapenemase-producing *Enterobacteriaceae* harboured a bla_{VIM}-type gene.
To conclude, the ongoing widespread diffusion of MDR bacteria in the LTCF suggests that efforts should be strengthened on MDR screening, implementation of infection control strategies and antibiotic stewardship programs targeting the unique aspects of LTCFs.

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INTRODUCTION

Long-term care facilities (LTCF) play an important role in contemporary healthcare systems due to an ageing population in industrialized countries, such as Italy where 22% of the population were over 65 years in 2015 (http://www.istat.it/). LTCFs provide ongoing skilled nursing care to residents in need of assistance with activities of daily living and help meet both the medical and non-medical needs predominantly of elderly people with a chronic illness or disability. Residents in these facilities have a variety of risk factors for colonization with multidrug-resistant (MDR) organisms.
Therefore these facilities represent reservoirs of *Enterobacteriaceae* expressing extended-spectrum β-lactamases (ESBLs), derepressed or acquired AmpC cephalosporinases, carbapenemases, methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE) (Moro *et al*., 2013; Cassone *et al*., 2015; Aschbacher *et al*., 2016). In 2008 and again in 2012 we undertook a point-prevalence survey for bacteria with these resistance phenotypes among residents and staff of a LTCF in Bolzano, Northern Italy, and among geriatric unit patients in the associated acute care hospital (March *et al*., 2010; March *et al*., 2014). The rationale for the repetition of the point prevalence screening study in 2016 in the same LTCF and hospital acute care unit was to determine the long-term trend in colonization prevalence with MDR bacteria of residents and staff, compared with geriatric unit patients of the associated acute care hospital.

Key words:
Long-term care facility, AmpC, ESBL, Carbapenemase, MRSA, VRE.

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MATERIALS AND METHODS

Facilities, resident and patient characteristics, survey design
In October 2016 we repeated a point prevalence study for MDR bacteria, first carried out in 2008 (March *et al*., 2010) and then repeated in 2012 (March *et al*., 2014), in a 120-bed LTCF that manages residents with different levels of independence, basal disease, comorbidity and functional status. All residents and staff were eligible to participate, as were patients in the 50 bed geriatric unit of the acute care hospital. The study was approved by the Ethics Committee of Bolzano Hospital.

Microbiological and molecular methods

Microbiological methods were similar to those used in the 2008 and 2012 studies (March *et al.*, 2010; March *et al.*, 2014), with minor modifications. Midstream or catheter urine samples, rectal, inguinal and oropharyngeal swabs from all participants were spread on Brilliance™ ESBL Agar (Oxoid Microbiology Products, Thermo Scientific, UK), applying a 10 µg imipenem disc (BIO-RAD, Marnes-la-Coquette, France), and on Liofilchem® Chromatic VRE (Liofilchem, Italy) agar plates, applying a 5 µg vancomycin disc (Becton Dickinson, USA); nasal swabs and the above mentioned samples except urine were also spread on BBL™ CHROMagar® MRSA II (Becton Dickinson, USA).

Isolates were identified by matrix-assisted laser desorption-ionization-time of flight (MALDI-TOF) Vitek MS mass-spectrometry (bioMérieux, France).

Antibiotic susceptibility testing was performed using the Vitek 2 System, calibrated against European Committee on Antimicrobial Susceptibility Testing criteria (www.EUCAST.org), with AST-N202 cards (including an ESBL test) for Gram-negative bacteria, AST-P632 cards (with both oxacillin and cefoxitin) for MRSA and AST-P586 cards for VRE.

Identification of β-lactamase types was based on Vitek 2 results, Etests (meropenem +/- EDTA for MBL in enterobacteria and imipenem +/- EDTA for MBLs in *Pseudomonas aeruginosa*, cefotaxime +/- clavulanate, ceftazidime +/- clavulanate and cefepime +/- clavulanate for ESBLs, bioMérieux) and the ESBL+AMPC Screen Kit and KPC+MBL Confirm ID Kit (ROSCO DIAGNOSTICA A/S, Denmark). Identification of $bla_{\text{CTX-M}}$-, bla_{SHV}-, bla_{TEM}- and bla_{VIM}-type genes was accomplished by PCR as previously described (Tzelepi *et al.*, 2003; Giakkoupi *et al.*, 2009).

Statistical analysis

The significance of differences in risk factors and colonization proportions was calculated by using the Chi-squared test or the proportion comparison test with Medcalc® version 15.11.4 (MedCalc software, Ostend, Belgium).

RESULTS

All 115 residents of the LTCF present in October 2016 participated in the point-prevalence study; 67 of the 91 (73%) LTCF staff also agreed to be enrolled, mainly nurses and physicians. The median age of LTCF residents was 83 years (range 24-96 years) and the percentage of women was 56%. All 50 acute care geriatric unit patients, with a median age of 85 years (range 74-98 years), joined the study; none was ordinarily a resident of the LTCF.

Table 1 shows demographic and clinical characteristics of LTCF residents and geriatric unit patients involved in the 2016 screening study. The median length of stay of residents in LTCF was 19 months (range <1-172 months).

As shown in *Table 2*, 66.1% of LTCF residents in 2016 were colonized by at least one resistant organism, as were 19.4% of LTCF staff members and 26.0% of geriatric unit patients; 63.4% of residents were colonized by extended-spectrum cephalosporin-resistant (ESCR) *Enterobacteriaceae*, predominantly ESBL-producers (53.0% of residents colonized, 7.8% by more than one species) and high-level AmpC-cephalosporinase producers (25.2% of residents colonized). Interestingly, high-level AmpC-producing *Morganella morganii* isolates from LTCF residents were highly prevalent (24.3%) in the 2016 screening study. Only two LTCF residents (1.7%) were colonized by carbapenemase-producing *Enterobacteriaceae*.

Table 1 - *LTCF resident and geriatric unit patient characteristics in the 2016 screening study.*

	LTCF residents colonized (%) Number of residents =115	*Geriatric unit patients colonized (%) Number of patients =50*
Male sex	43.4	48.0
Age ≥ 86 years	35.6	48.0
Antibiotics in last 3 months	23.4	58.0
Fluoroquinolones	5.2	14.0
Penicillins	12.1	38.0
Cephalosporins	1.7	6.0
Dementia	68.7	62.0
Peripheral vascular disease	71.3	98.0
Incontinence	85.2	76.0
Diabetes	20.8	22.0
Cancer	9.5	20.0
Decubitus ulcer	11.3	0.0
Chronic obstructive pulmonary disease	18.2	22.0
Physical disability (Barthel immobility score of 0)	67.8	
Apallic state (coma)	17.4	0.0
Any medical device	38.2	24.0
Percutaneous enteral gastrostomy tube	20.8	0.0
Tracheostomy tube	9.5	0.0
Urinary catheter	18.2	24.0
Nasogastric tube	1.7	0.0

Table 2 - *Percentages of colonization in residents and staff from the LTCF and geriatric unit patients.*

	LTCF residents colonized (%)	*LTCF staff colonized (%)*	*Geriatric unit patients colonized (%)*	*p-value for differences between LTCF residents and geriatric unit patients*
	Number of residents = 115	*Number of staff members = 67*	*Number of patients = 50*	
All resistance groups (MRSA, VRE and enterobacteria ESBL, AmpC, MBL)	66,1%	19,4%	26,0%	<0.001
All enterobacteria, ESBL-positive	53,0%	11,9%	12,0%	<0.001
Escherichia coli, ESBL-positive	45,2%	11,9%	8,0%	<0.001
Proteus mirabilis, ESBL-positive	7,0%	0,0%	0,0%	0.05
Klebsiella pneumoniae, ESBL-positive	6,1%	0,0%	4,0%	0.58
Morganella morganii, ESBL-positive	2,6%	0,0%	0,0%	0.25
Citrobacter koseri, ESBL-positive	0,8%	0,0%	0,0%	0.52
Citrobacter freundii, ESBL-positive	0,0%	0,0%	2.0%	0.19
Alle enterobacteria, high-level AmpC	25,2%	0,0%	8,0%	0.01
Enterobacter cloacae, high-level AmpC	0,0%	0,0%	4,0%	0.03
Morganella morganii, high-level AmpC	24,3%	0,0%	0,0%	<0.001
Citrobacter freundii, high-level AmpC	0,0%	0,0%	4,0%	0.03
Proteus mirabilis, high-level AmpC	0,8%	0,0%	2,0%	0.51
All enterobacteria, MBL-positive	1,7%	0,0%	4,0%	0.37
MRSA	14,8%	7,4%	6,0%	0.11
VRE	0,8%	0,0%	0,0%	0.52

PCR testing showed that 80.3% of *Escherichia coli* isolates from LTCF residents had $bla_{CTX\text{-}M}$-type genes, 15.1% harbored bla_{SHV}-type genes and 83% had a bla_{TEM}-type determinant. The bla_{VIM}-type gene was present in one *E. coli* isolate, together with a bla_{TEM}-type determinant (corresponding to 1.9% of isolates from LTCF residents). Moreover, all *E. coli* isolates from LTCF staff had $bla_{CTX\text{-}M}$-type genes. Four out of five *E. coli* isolates collected from geriatric unit patients had a $bla_{CTX\text{-}M}$-type gene; in the remaining *E. coli* strain, a bla_{VIM}-type gene was detected. By PCR testing 62.5% of *Klebsiella pneumoniae* isolates from LTCF residents and all *K. pneumoniae* from geriatric unit patients had $bla_{CTX\text{-}M}$-type genes; one of the latter isolates, besides the $bla_{CTX\text{-}M}$-type gene, also harboured bla_{VIM}. Finally, no $bla_{CTX\text{-}M}$-type determinant was detected in the 31 *M. morganii,* nine *Proteus mirabilis*, five *Citrobacter spp*. and three *Enterobacter spp.* The single isolate of *Citrobacter amalonaticus* from a LTCF resident and the unique *Enterobacter aerogenes* strain from a geriatric unit patient (who was also colonized by the bla_{VIM} producing *K. pneumoniae*) harboured a bla_{VIM}-type gene together with a $bla_{SHV\text{-}12}$ determinant.

Table 3 - *Percentages of residents to be positive for methicillin-resistant Staphylococcus aureus (MRSA) or Enterobacteriaceae-producing extended-spectrum* β*-lactamase (ESBL), derepressed or acquired AmpC-cephalosporinases or metallo-*β*-lactamases (MBLs) with various specimen type combinations.*

	MRSA (%)	*ESBL, AmpC or MBL producing Enterobacteriaceae (%)*
Rectal	17	90
Inguinal	23	79
Oropharyngeal	88	15
Nasal	47	
Urina		52
Rectal+inguinal	29	100
Rectal+oropharyngeal	94	90
Rectal+nasal	53	
Rectal+urine		98
Inguinal+oropharyngeal	88	84
Inguinal+nasal	53	
Inguinal+urine		89
Oropharyngeal+nasal	94	
Oropharyngeal+urine		58

MRSA colonization prevalence was 14.8% in LTCF residents, 7.4% in LTCF staff and 6.0% in geriatric unit patients. Colonization of residents with VRE was rare (0.8%). No LTCF staff member was colonized with *Pseudomonas aeruginosa*, whereas 75.6% of LTCF residents (rectal = 62%, inguinal = 35%; urine = 20%; oropharyngeal = 20%) and 32% of geriatric unit patients were colonized; none of these isolates was a MBL producer. No *Acinetobacter baumannii* grew on the Brilliance ESBL Agar (an OXA-23 carbapenemase producing strain was used as positive control).

Enterobacteriaceae producing ESBLs, derepressed or acquired AmpCs or MBLs in LTCF residents were most often recovered from rectal swabs (90%) and MRSA from oropharyngeal (88%) specimens (*Table 3*). The best screening strategies to detect colonized residents required both rectal and inguinal samples for *Enterobacteriaceae* and oropharyngeal, combined with nasal or rectal samples for MRSA.

DISCUSSION

In 2016, we repeated a screening study, first undertaken in 2008 (March et al., 2010) and then repeated in 2012 (March *et al.*, 2014), for MDR *Enterobacteriaceae*, MRSA and VRE in a LTCF and the associated acute care hospital geriatric ward. Comparing data from the 2016 screening study with data from the two previous studies, 66.1% of LTCF residents in 2016 were colonized by at least one resistant organism, compared with 74.8% in 2008 (p=0.15) and 53.8% in 2012 (p=0.06); 19.4% of LTCF staff members in 2016, compared with 27.5% in 2008 (p=0.26) and 10.5% in 2012 (p=0.17) were also colonized, as were 26.0% of geriatric unit patients in 2016, compared with 22.2% in 2008 (p=0.66) and 22.7% in 2012 (p=0.71).

Differences in LTCF resident colonization prevalence with ESBL producing *Enterobacteriaceae* between 2016 (53.0%) and 2008 (64.0%) or 2012 (49.0%) were not statistically significant (p-values of 0.09 and 0.55, respectively), as were not significant differences for LTCF staff or geriatric unit patients.

On the other hand, LTCF resident colonization prevalence for high-level AmpC-producing *Enterobacteriaceae* was significantly higher (p<0.001) in 2016 (25.2%) compared with 2008 (4.5%) and 2012 (3.8%); this difference was caused by a significant increase (p<0.001) of high-level AmpC-producing *Morganella morganii* in 2016 (24.3%), according to phenotypic tests, compared with 2008 (0.0%) and 2012 (1.9%). Only two LTCF residents (1.7%) were colonized by carbapenemase-producing *Enterobacteriaceae* in 2016.

Colonization prevalence of LTCF residents with MRSA was significantly lower in 2016 (14.8%) compared with 2008 (38.7%; p<0.001), but it was similar in 2012 (13.2%; p=0.73). A striking feature is the colonization of 19.4% of LTCF staff with resistant bacteria in 2016 (ESBL: 11.9%; MRSA: 7.4%), compared with 27.5% in 2008 (ESBL: 14.5%; MRSA: 14.8%) and 10.5% in 2012 (ESBL: 5.2%; MRSA: 7.0%). This carriage probably reflects resident-to-staff transmission facilitated by the low functional and cognitive status and the generally low mobility score of LTCF residents, requiring continuous nursing care for daily living activities, with many occasions for horizontal transmission of MDR organisms between residents and health care workers.

Differences in colonization prevalence between LTCF residents and geriatric unit patients are highly significant (p<0.001) for ESBL and high-level AmpC-producing *Enterobacteriaceae*. Similarly to colonization rates in LTCF residents, percentages of acute care hospital's inpatient MRSA isolates (2008: 21.8%; 2012: 18.9%; 2016: 20.3%) or of ESCR *E. coli* isolates (2008: 10.9%; 2012: 12.8%; 2016: 10.0%) did not change significantly (p=0.64 and p=1.00, respectively). On the other hand, ESCR isolates of *K. pneumoniae* increased significantly (2008: 12.2%; 2012: 16.7%; 2016: 29.2%; p<0.001), but the increase in this resistance phenotype in the acute care hospital was not paralleled by increasing colonization prevalence of the same phenotype in LTCF residents (data for 2008 and 2012: March *et al.*, 2014; data for 2016: Aschbacher R, unpublished data).

In 2016, in our LTCF, 63.4% and 14.8% of residents were colonized by extended-spectrum cephalosporin-resistant enterobacteria and MRSA, respectively. Slightly lower colonization rates of 56.0% and 12.0%, respectively, were found in 2012 in a second LTCF in the same Bolzano health district (March *et al.*, 2014). In 2006, a mean colonization percentage of 54% for residents bearing a urinary catheter in 23 Italian LTCFs (Arnoldo *et al.*, 2013) was reported, whereas in 2015 in four Italian cities on average 57.3% of residents were colonized by ESBL-producing enterobacteria and 17.2% by MRSA (Giufrè *et al.*, 2017). Nasal MRSA carriage of 7.8% and 19.3%, respectively, was found in 2006 in residents of two Italian LTCFs (Brugnaro *et al.*, 2009) and in 2005 in residents of one LTCF (Monaco *et al.*, 2009), respectively; the last study found a MRSA prevalence for staff of 5.8%. In various screening studies in other European countries, a high variability of colonization frequencies for ESBL-producing *Enterobacteriaceae* and MRSA, ranging from close to 0% up to levels higher than 50% were found; variability is high especially among countries but also among different LTCFs within single countries (Aschbacher *et al.*, 2016). As shown for ESBL-producing *E. coli* isolates from LTCF residents and staff from the two previous screening studies in the same LTCF (March *et al.*, 2010; March *et al.*, 2014), a high predominance of CTX-M-type enzymes was also found in 2016; isolates from the two previous screening studies were mainly *E. coli* ST131 expressing CTX-M-15-like enzymes. VRE colonization in our LTCF in 2016 was low (0.8% of residents), similarly to 2008 (2.7%) and to other European studies in LTCFs (March *et al.*, 2010, Aschbacher *et al.*, 2016).

In 2016, in our LTCF, colonization of residents by carbapenemase-producing enterobacteria was low (1.7%) and no staff member was colonized. A similarly low, mean colonization rate (1.0%) was found in LTCF residents of various Italian Provinces in 2015 (Giufrè *et al.*, 2017). The two carbapenemase producers in our study expressed VIM-type enzymes, predominant in the Bolzano healthcare district (Carattoli *et al.*, 2010; Aschbacher *et al.*, 2011; Aschbacher *et al.*, 2013). Other authors found VIM-type carbapenemases (Accogli *et al.*, 2014; Giufrè *et al.*, 2017), *K. pneumoniae* carbapenemases (KPCs) (Del Franco *et al.*, 2015; Piazza *et al.*, 2016; Giufrè *et al.*, 2017) and New Delhi metallo-β-lactamases (NDMs) (Gaibani *et al.*, 2011) in screening or clinical isolates from Italian LTCFs. The low colonization prevalence of carbapenemase-producing *Enterobacteriaceae* in Italian LTCF residents is surprising because Italy has one of the highest prevalence rates of this resistance phenotype in blood isolates from Europe (Annual report EARS-Net, 2015).

Much lower colonization rates with MDR bacteria in the screening studies were found in the Bolzano geriatric unit of the acute care hospital compared with LTCF residents. The carbapenemase-producing *Enterobacteriaceae* isolates (1 *E. coli*, 1 *K. pneumoniae*, 1 *E. aerogenes*) from geriatric unit patients harboured bla_{VIM}-type genes, similar to previous studies (March *et al.*, 2010; March *et al.*, 2014). No significant differences in colonization frequencies were found for geriatric unit patients between the screening studies in 2008, 2012 and 2016, though significant differences were found for percentages of male patients (2008: 28.9%, 2012: 27.3%, 2016: 48.0%) and dementia patients (2008: 33.3%, 2012: 31.8%, 2016: 62.0%), significantly higher in 2016 compared with 2008 (p=0.05 and p=0.05, respectively) and 2012 (p=0.04 and p=0.003, respectively).

Usage of an oropharyngeal swab for screening of LTCF

residents for MRSA and a rectal swab for screening of enterobacteria-producing ESBLs, high-level AmpCs or carbapenemases, gave recovery rates of 88% and 90%, respectively (compared with the combination of all four specimen types). Recovery rates for MRSA from oropharyngeal swabs in 2016 are in contrast with the two previous screening studies in 2008 and 2012 in the same LTCF where recovery rates of only 50% and 72%, respectively, were obtained (March *et al.*, 2010; March *et al.*, 2014). Moreover, using nasal swabs alone we would have obtained MRSA recovery rates of only 45%, 52% and 47% in 2008, 2012 and 2016, respectively. In many Italian and other European colonization studies, the only sample type for MRSA screening was nasal swabs, leading to possible significant underestimation of real colonization frequencies. On the other hand, using only rectal swabs for ESBL screening, as done in most of the colonization studies, still permits high recovery rates of 77-96% (Aschbacher *et al.*, 2016).

Few risk factors for colonization with MDR bacteria in LTCF residents or geriatric unit patients changed significantly from 2008 (March *et al.*, 2010) or 2012 (March *et al.*, 2014) to 2016. The number of coma residents in the LTCF was lower in 2012 (10 residents) compared with 2008 and 2016 (20 residents each), the percentage of catheterized residents was also lower in 2012 (24.5%) compared with 2008 (48.6%; p<0.001) and 2016 (38.2%; p=0.03), whereas the percentage of dementia residents in 2012 (85.8%) was significantly higher compared with 2008 (66.7%; p=0.002) and 2016 (68.7%; p=0.002). Chronic obstructive pulmonary disease increased significantly in 2016 (18.2%) compared with 2008 (6.3%; p=0.006). The lower number of coma residents and catheterized residents and the higher percentage of dementia residents in 2012 compared with 2008 and 2016 could be a partial explanation for the lower colonization rates by MDR bacteria in 2012 compared with 2008 (p=0.002) and for the marginally lower rates compared with 2016 (p=0.06). Nevertheless, strengthened hygiene measures introduced after the 2008 screening, according to World Health Organization guidelines (WHO guidelines on hand hygiene, 2009), were maintained throughout the period 2009-16. Therefore, similar colonization rates in 2016, compared with the previous studies, confirm that control of colonization by MDR organisms in LTCFs is challenging (Smith *et al.*, 2008). Nevertheless, the snapshot approach (samples were collected only once in 2008, 2012 and 2016, respectively, in a short period of time) might lead to the sudden increase in prevalence of specific resistance phenotypes, as shown for high-level AmpC-producing *M. morganii* in 2016 which could be a transient phenomenon, and the choice of a point prevalence study might therefore explain the discrepancy in the conclusions of the two studies (preventive measures seemed to be effective in 2012 but not in 2016).

This study has some limitations. First, it was carried out in a single LTCF, not allowing for extrapolation of data to other LTCFs in other Italian cities. Second, molecular characterization of isolates in the 2016 study was limited. Despite these limitations, the strength of our study is the 100% participation of LTCF residents, the comparison with 100% of geriatric unit patients present in the associated acute care hospital unit and the inclusion of a high percentage of LTCF staff in the screening survey. Moreover, the repetition of the screening in four-year intervals (2008, 2012 and 2016) allows evaluation of a long-term trend in colonization frequencies.

The ongoing widespread diffusion of MDR bacteria in LTCFs in the Bolzano healthcare area confirms that LTCFs are a potentially important reservoir for MDR organisms and suggests that future efforts should focus on MDR screening, improved implementation of infection control strategies and antibiotic stewardship programs targeting the unique aspects of LTCFs. To promote further studies of various microbiological aspects related to LTCFs, the Association of Italian Clinical Microbiologists (Associazione Microbiologi Clinici Italiani; AMCLI) in 2016 has set up a new working group consisting of Clinical Microbiologists (Gruppo di Lavoro per lo Studio delle Infezioni nelle Residenze Sanitarie Assistite e Strutture assimilabili; GLISTer); the present study was supervised by this working group.

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Conflict of interest

All authors: nothing to declare.

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