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Inguinal skin colonization with multidrug-resistant bacteria among residents of elderly care facilities: Frequency, persistence, molecular analysis and clinical impact

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ABSTRACT

Frequency, persistence and molecular characteristics of multidrug resistant bacteria colonizing inhabitants of long term care facilities are topics of current concern. We performed a point-prevalence survey of 402 residents in 7 elderly care facilities in Berlin, Germany. Inguinal swabs were analyzed for the presence of methicillin-resistant Staphylococcus aureus (MRSA), vancomycin-resistant Enterococci (VRE), and multidrug-resistant gram-negative bacteria. Three and six months following the initial investigation, all colonized residents were sampled again and the occurrence of intercurrent infections, hospital admissions and use of antimicrobials were registered. Genetic relatedness of the bacteria was investigated using multi-locus sequence typing (MLST), spa-typing and SmaI/XbaI-macrorestriction analysis. 33 (8.2%) residents were skin-colonized with multidrug-resistant bacteria. MRSA were found in 19 (4.7%) and ESBL-producing Enterobacteriaceae in 16 residents (3.98%). Independent risk factors for colonization with multidrug-resistant bacteria were a high level of care and the presence of chronic wounds. A large proportion of the observed bacteria persisted up to six months and showed a high degree of inter-individual diversity. Outcome analysis revealed that infections tend to occur slightly more often in residents colonized by multiresistant pathogens. We assume that a perceptible population of residents in nursing homes is at risk for individual colonization with multidrug-resistant bacteria as well as healthcare associated infections.

Introduction

Current trends in the demographic structure in Germany indicate increasing requirements in long term care. In recent years, elderly care facilities and nursing homes had to face progressively the issues of multidrug-resistant bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci (VRE), *Enterobacteriaceae* with resistance to third generation cephalosporins and multidrug-resistant nonfermenting species like *Pseudomonas aeruginosa* and *Acinetobacter baumannii* (Barr et al., 2007; Benenson et al., 2009; Gruber et al., 2013; O'Fallon et al., 2009b). These facilities are forced to manage different aspects of collective living of a population uniquely prone to infectious diseases, coping with immunosenescence, comorbidities and polypharmacy (Yoshikawa, 2000).

Asymptomatic colonization by multidrug-resistant bacteria in this population not only includes the risk of subsequent infection in the individual, but also implies a potential source of transmission. Several studies suggest a possible reservoir of multidrug-resistant bacteria in long term care associated with recent antibiotic use, previous hospitalization and immobility (Gruber et al., 2013; March et al., 2009; Rooney et al., 2009). However, little is known about the persistence and clinical significance of asymptomatic colonization among residents of elderly care facilities. An influence of colonization on increased mortality and morbidity is assumed at least for MRSA carriers (Datta and Huang, 2008; Suetens et al.,

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2006). However, the significance of asymptomatic colonization with multidrug-resistant gram-negative bacteria in long term care remains unclear so far.

Our study aimed to investigate skin carriage of multidrug-resistant bacteria in long term care facilities, as a relevant site for healthcare associated bacterial transmission. In order to get a comprehensive insight into the potentially altered inguinal skin colonization of our study population we broadened the spectrum of target organisms and included *Candida* spp. as well as gram negative non-enterobacteriaceae displaying resistance to cefpodoxim. Furthermore, we investigated the duration of inguinal colonization over a period of six months and molecular typing of isolated strains The comparison of clinical outcomes of colonized versus non-colonized residents should shed light on the clinical impact of colonization in residents of elderly care facilities.

Materials and methods

Study design and setting

A cross sectional point prevalence survey was conducted in seven long term care facilities in Berlin, Germany between May and September 2011. We included only voluntarily participating elderly care facilities, addressed by local health authorities; specialized care facilities or rehabilitation clinics were excluded. Following the initial survey, participating residents were attended over a period of six months and microbiological sampling of initially colonized residents was repeated three and six months after the initial survey. Structured questionnaires were completed and medical records were screened for all eligible residents at the initial survey and at both follow-up screenings.

Ethical statement

The study protocol was approved by the local Ethics Committee. Residents and their relatives as well as general practitioners of the facilities were provided with written information about the purpose and process of the study. Written consent was obtained from all residents or from legal representatives in case of cognitive disabilities. Besides, residents and their relatives were informed during information events in the facilities and all were given the possibility to ask about the aims of the study and the issue of multidrug-resistant bacteria. Nursing staff and healthcare workers were trained how to obtain microbiological swabs and how to deal with colonized residents in their facilities in terms of infection control measures and standard precautions.

Data collection and analysis

At the initial survey, a structured questionnaire was completed for each participant in order to obtain demographic and administrative data as well as data concerning possible risk factors for asymptomatic colonization by multidrug-resistant bacteria. Medical records of eligible residents were reviewed and local nursing staff assisted in data collection to complete the questionnaires. Collected variables included: age, gender, length of stay in the facility, accommodation (single, double or shared rooms), level of care, mobility, incontinence, dementia or cognitive disorders, previous hospitalization and surgeries (in the past three months), previous antibiotic use (in the past three months), presence of gastrostomy and/or urinary catheters, presence of chronic wounds (decubitus ulcers, surgical wound, trauma), previously known colonization with multidrug-resistant bacteria, diabetes mellitus and whether haemodialysis is required. Autonomy in basic activities of daily living was measured by the Barthel-index-score, where 100 points represent a high level of autonomy in basic activities and 0 points a high level of dependency in activities of daily living (bathing, dressing, eating, going to the toilet, walking and continence).

Questionnaires for the two follow-up surveys after three and six months included type of colonization at initial survey, antibiotic treatment, information on decolonization (for MRSA colonized residents) hospitalization and any kind of infection during the past three months (if available with microbiological results) as well as cases of death.

Microbiological diagnostics and analysis

Microbiological samples of all residents enrolled in the study were collected by local nursing staff at the day of the point prevalence survey. In all facilities, sampling was scheduled in the morning ward round, before bathing and dressing of residents. Specimens were taken using one culture swab (Becton Dickinson Cultureswab, Amies-medium without charcoal) for both groins of each eligible resident.

Each swab was solved in 1 ml phosphate buffered saline (PBS) of which 50 μl were plated on columbia agar (Oxoid) charged with 5% defibrinated sheep blood, Endo agar (Biomérieux) and different chromogenic selective media (MRSA: CHROMagar MRSA, Becton Dickinson; ESBL: chromID ESBL, Biomérieux; VRE: chromID VRE, Biomérieux; *Candida* spp.: chromID candida, Biomérieux), respectively. Growth characteristics and haemolysis were evaluated after aerobic incubation at 36 °C, first after 18 h and a second time after 36 h. Suspected colonies growing on selective media as described by the manufacturer were further cultivated on Mueller-Hinton sheep blood agar and identified to species level using the automated Vitek-2 system (Biomérieux).

Microbiological analysis of MRSA

S. aureus detection was based on colony morphology, haemolysis and latex agglutination test directed against capsular antigens serotype 5 and cell wall antigens (Pastorex™ Staph Plus, BioRad).

Antimicrobial susceptibilities were determined by using the microbroth dilution method according to DIN58940 and applying breakpoints of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) for interpretation of the results (http://www.eucast.org). The MIC test panel included penicillin G, oxacillin, gentamicin, erythromycin, clindamycin, ciprofloxacin, moxifloxacin, tetracycline, cotrimoxazol, rifampicin, fusidic acid, fosfomycin, linezolid, mupirocin, daptomycin, tigecyclin, vancomycin, and teicoplanin.

DNA was extracted from an overnight culture using a DNeasy tissue kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions with the modification that lysostaphin (100 μg/ml; Sigma, Munich, Germany) was added to the cell-lysis step. PCR detection of the *mecA*-gene was done as published earlier (Strommenger et al., 2003). Spa-typing was performed by amplifying and sequencing of the polymorphic X-region of the protein A gene (spa) according to the Ridom StaphType standard protocol (www.ridom.com). The resulting spa-types were assigned by using the Ridom StaphType software (Ridom GmbH, Würzburg, Germany). Multilocus sequence typing (MLST) for *S. aureus* was done as described elsewhere (Enright et al., 2000). The HARMONY protocol was used for obtaining PFGE patterns of *Sma*I-digested genomic DNA (Cookson et al., 2007). The resulting patterns were analyzed using the Bionumerics software (Applied Maths, Ghent, Belgium).

Microbiological analyses of gram-negative bacteria

Antimicrobial susceptibilities of gram-negative bacteria were tested using the VITEK-card AST-N110 (Vitek-2, Biomérieux) with

interpretation of the results according to EUCAST breakpoints (http://www.eucast.org/clinical_breakpoints). Enterobacterial isolates with resistance to cefotaxime and/or ceftazidime were screened for presence of ESBL genes ($bla_{TEM\text{-}type}$, $bla_{SHV\text{-}type}$ and $bla_{CTX\text{-}M\text{-}1\text{-}2\text{-}9\text{-}type}$) and *ampC* genes ($bla_{CMY\text{-}type}$, $bla_{DHA\text{-}type}$) by PCR and sequencing (Gröbner et al., 2009; Pfeifer et al., 2009). All carbapenem-non-susceptible gram-negative isolates were tested for carbapenemase production (mastdiscs™ *combi* Carbapenemase Detection Set; Modified Hodge test) according to standard protocol CLSI (Clinical and Laboratory Standards Institute C., 2012) and presence of different carbapenemase genes ($bla_{VIM\text{-}type}$, $bla_{KPC\text{-}type}$, $bla_{NDM\text{-}type}$, $bla_{IMP\text{-}type}$, $bla_{GIM\text{-}type}$, $bla_{OXA\text{-}48\text{-}type}$) as described previously (Gröbner et al., 2009; Pfeifer et al., 2011).

Basic typing of all ESBL-*Escherichia coli* isolates was performed by determination of the four *E. coli* phylogenetic groups (A, B1, B2 and D) using a PCR-based assay (Clermont et al., 2000). Furthermore, for all ESBL-producing isolates *Xba*I-macrorestriction and subsequent pulsed-field gel electrophoresis (PFGE) with interpretation of genetic relationship according to the criteria of (Tenover et al., 1995) were performed.

Statistical analysis

Fisher's exact test was performed for degree of association for categorical variables and Welch's two sample *t*-test was performed for comparison of mean values of age and Barthel-index score between colonized and non-colonized residents. Odds ratios and 95% confidence intervals are given. All tests were performed two-tailed and a p-value <0.05 was considered significant. All significant predictors of univariate analysis were further included into the multivariate model using logistic regression with backward elimination (criterion: $\alpha = 0.05$). The dependent variable was defined as the presence or absence of one of each isolated microorganism of interest, or both together (MRSA and ESBL).

Outcome comparison of the follow-up screenings was performed using the chi square test (p-value <0.05). In order to reveal potential confounders in the outcome analysis, a multivariate analysis of risk factors for infections in the study population was performed.

Results

Initial point prevalence survey

Study population and case mix

Seven elderly care facilities with a total of 864 residents agreed to participate and were enrolled in the study. Overall participation rate of residents was 46.5% ($n = 402$) and ranged between 34.4% and 75.5% in the seven facilities. Distribution of care levels as an indicator of care intensity was approximately the same between eligible residents and those who refused to participate. Demographic characteristics of eligible residents are summarized in Table 1.

Antibiotic treatment

Systemic antibiotic treatment during the past three months was registered in 38 of the residents (9.5%), 22 of them received the antibiotic therapy for more than one week (5.5%). Prevalence of previous antibiotic treatment ranged between 4.9% and 21.7% within the seven facilities (see Table 1). Fluorquinolones were the most commonly prescribed antimicrobial agents (28%), followed by cotrimoxazole (10%), combinations of penicillins including beta-lactam/beta-lactamase inhibitor combinations (9%) as well as third and second generation cephalosporins (each 8%). Documented indications for antibiotic treatment included symptomatic urinary tract infections (42%), upper respiratory tract infections (10%) followed by bronchitis/tracheobronchitis (7%). In a significant amount of

Table 1
Characteristics of eligible residents at initial survey (%).

Nursing home	Residents	Eligible residents																	
	n	%	Care level			Age (median)	Female	Hospitalization[a]	Antibiotic usage[b]	Dementia	Incontinence		Bed-ridden	Wheel-chair	Ambulant	Diabetes mellitus	Wounds	Dialysis	Tracheostomy	Urinary catheter	Gastrostomy
			0 and 1	2	3 and 3+						Bowel incontinence	Bladder incontinence									
101	238	34.5	28	32.9	39	85	72	2.4	4.9	80	50	68.3	18	41	40.2	18.3	12.2	1.2	1.2	2.4	8.5
201	64	34.4	22.7	45.5	31.8	87.5	86	9.1	13.6	82	50	63.6	14	32	54.5	22.7	0	0	0	0	4.5
301	94	75.5	45.1	38	16.9	88	86	4.2	9.9	63	29.6	59.2	1.4	39	59.2	31	5.63	0	0	0	0
401	88	55.7	40.8	44.9	14.3	90	88	10	6.1	73	40.8	71.4	2	51	46.9	8.16	12.2	0	0	4.1	0
402	100	46	54.3	34.8	10.9	88	83	11	21.7	48	21.7	60.9	6.5	41	52.2	17.4	8.7	0	0	6.5	4.3
501	160	50	50	26.3	23.8	85	79	19	7.5	59	31.3	67.5	11	34	55	21.3	10	0	0	2.5	2.5
601	120	43.3	44.2	30.8	25	86	79	9.6	9.6	69	36.5	57.7	9.6	29	61.5	23.1	13.5	0	0	3.8	3.8
Total	864	46.5	42	34.6	23.6	87	81	9.2	9.5	67	36.6	64.4	9.2	39	52.2	20.6	9.7	0.2	0.2	2.7	3.5

[a] Inpatient admission to an acute care hospital during the last three months.
[b] Systemic antibiotic usage during the last three months.

Table 2
Prevalence rates of residents colonized by MRSA, ESBL-producing Enterobacteriaceae, nonfermenting gram-negative bacteria and Candida spp. at initial survey.

Nursing home	Number of ER[a]		MRSA		ESBL-producing *Enterobacteriaceae*		Other gram-negative bacteria		Candida spp.	
	n	%	*n*	%	*n*	%	*n*	%	*n*	%
101	82	34.5	5	6.1	3	3.7	8	9.8	6	7.3
201	22	34.4	1	4.5	0	0.0	1	4.5	3	13.6
301	71	75.5	1	1.4	3	4.2	7	9.9	8	11.3
401	49	55.7	2	4.1	1	2.0	3	6.1	7	14.3
402	46	46	5	10.9	2	4.3	7	15.2	3	6.5
501	80	50	5	6.3	3	3.8	11	13.8	6	7.5
601	52	43.3	0	0.0	4	7.7	9	17.3	5	9.6
Total	402	46.5	19	4.7	16	4.0	46	11.4	38	9.5

[a] Eligible residents.

antibiotic prescriptions (13%), indications for antibiotic treatments were only poorly or not documented at all. For two residents that were recently discharged from acute care hospitals, the documented indication for the prescription of antibiotic treatment (for both with carbapenems) was the presence of ESBL producing bacteria.

Prevalence of MRSA

At the initial survey, 18 residents were shown to have inguinal skin colonization with MRSA (4.7%). Prevalence rates of MRSA ranged between 0% and 10.9% in the seven facilities (Table 2). Isolated MRSA mainly revealed *spa* type t032 (57.9%), belonging to clonal complex 22. Results of genotyping and phenotypic resistance of the isolated MRSA are summarized in Table 4. Almost all isolated MRSA (94.7%) displayed co-resistance to ciprofloxacin and moxifloxacin. Resistance towards erythromycin/clindamycin was detected in 52.6% of the isolated MRSA. One isolate (ST225, t003) displayed mupirocin-resistance and another one showed an intermediate resistance phenotype to mupirocin (CC22, t492).

Prevalence of ESBL-producing Enterobacteriaceae

We detected 16 residents harbouring ESBL-producing Enterobacteriaceae (4.0%). Prevalence ranged between 0% and 7.7% in the seven participating facilities (Table 2). Two residents were co-colonized with both, MRSA and ESBL-producing Enterobacteriaceae, therefore yielding an overall prevalence of multidrug-resistant bacteria of 8.21% (32 residents). Results of genotyping and identification of β-lactamase-genes are summarized in Table 5.

Co-resistance to ciprofloxacin was found in 56.3% of the ESBL-producing *Enterobacteriaceae*, Carbapenem resistance was not observed.

Prevalence of other bacterial species

A total of 49 other (non Enterobacteriaceae) gram-negative strains were isolated from 46 residents, reflecting an overall prevalence of 11.4% including *Pseudomonas aeruginosa* *n* = 31, 63.3%; *Stenotrophomonas maltophilia* *n* = 9, 18.37%; *Acinetobacter ursingii* *n* = 1, 2%; *Acinetobacter baumannii* *n* = 2, 4.1%; *Achromobacter denitrificans* *n* = 1, 2%; *Pseudomonas fluorescens* *n* = 1, 2%; *Pseudomonas putida* *n* = 2, 4.1%; *Aeromonas salmonicica* *n* = 1, 2% and *Sphingobacter multivorum* *n* = 1, 2%. One resident was co-colonized with *Acinetobacter baumannii* and *Pseudomonas aeruginosa* and another one with *Aeromonas salmonicica*; *Pseudomonas putida* and *Stenotrophomonas maltophilia*. Prevalence rate of gram-negative bacteria (non Enterobacteriaceae) ranged from 4.6% to 17.3% in the seven facilities (see Table 2).

In total, six *P. aeruginosa* isolates (19.4%) were resistant to ceftazidime and five to ciprofloxacin (16.1%). Furthermore, ten isolates were resistant or intermedia resistant to meropenem and/or imipenem including three isolates that were additionally resistant to ciprofloxacin and one isolate which was resistant to ciprofloxacin and ceftazidime. Carbapenemase genes were not identified in these strains.

Prevalence of Vancomycin-resistant Enterococcus spp.

Regarding gram-positive pathogens, Vancomycin-resistant enterococci were not found, neither at initial survey nor in the follow-up screenings.

Prevalence of Candida spp.

Candida spp. was isolated in 38 residents (*Candida albicans* *n* = 24; 63.2%; *Candida krusei* *n* = 16, 42.1%), representing a prevalence of 9.5%. Three residents were co-colonized with both species at the same time. Range of prevalence rates was 6.5% to 14.3% within the seven facilities (Table 2).

Risk factor analysis

Significant risk factors for colonization with multidrug-resistant bacteria (ESBL or MRSA) in univariate analysis are summarized in Table 3. Independent risk factors for inguinal colonization with MRSA and/or ESBL-producing bacteria as results of logistic regression were a high level of care (*p*-value: <0.001) and the presence of wounds (*p*-value: <0.001). For MRSA carriage, multivariate analysis also revealed a known history of multidrug-resistant bacteria as an independent predictor (*p*-value <0.001). ESBL carriage was associated with a high level of care (*p*-value: 0.001), the presence of wounds (*p-value 0.01*), percutaneous gastrostomy (*p*-value: 0.05) and faecal incontinence (*p*-value: 0.05).

The only independent risk factor for inguinal skin colonization with other gram-negative bacteria (*Pseudomonas* spp., *Acinetobacter* spp.) was immobility (*p*-value <0.001).

First follow-up (3 months after)

MRSA and ESBL-colonization

Three months following the initial survey, MRSA were detected again in 9 of the initially 19 colonized residents (47.3%). In one resident, initially proven to be colonized by an ESBL-*E. coli*, the first follow-up screening also detected a MRSA. Medical records of all MRSA-colonized residents were reviewed for information concerning MRSA-decolonization treatment after the initial survey. However, documentation (incl. kind of treatment, duration and used agents) was rather poor and applied schemes varied widely, even within the same facility. Duration of performed treatments was not documented at all. According to information of health care staff and nurses in the wards, 15 of the initially 19 MRSA-colonized residents underwent a decolonization treatment (78.9%) during the three months before the first follow-up. Table 4 provides an overview of MRSA colonization and persistence in the colonized residents.

Table 3
Univariate risk-factor analysis for inguinal colonization with MRSA or ESBL-producing bacteria.

	MRSA				ESBL			
	MRSA		No MRSA		ESBL		No ESBL	
	n	%	*n*	%	*n*	%	*n*	%
n (%)	**19**	**4.7**	**383**	**95.3**	**16**	**4.0**	**386**	**96.0**
Characteristics								
Welch's two sample *t*-test								
Age								
Mean value	84.8		85.3		79.8		85.5	
Median	86.5		87.0		82.0		87.0	
	95% CI		*p*-Value		95% CI		*p*-Value	
	−4.13 to 5.07		0.831		1.26–11.01		0.017	
Barthel index score								
Mean value	37.2		49.6		26.8		50.0	
Median	37.5		50.0		22.5		50.0	
	95% CI		*p*-Value		95% CI		*p*-Value	
	−25.69 to 0.94		0.067		**−37.94 to −8.59**		**0.004**	
Fishers exact test								
	OR	95% CI	*p*-Value		OR	95% CI	*p*-Value	
Known history of MDRB	**8.41**	**2.09–29.34**	**0.002**		4.68	0.79–19.33	0.044	
MRSA	5.24	0.87–21.90	0.035		3.51	0.36–17.65	0.143	
ESBL	**46.05**	**2.29–2765.27**	**0.006**		12.55	0.20–253.40	0.115	
VRE	–	–	–		–	–	–	
Previous antibiotic treatment	1.93	0.34–7.30	0.402		**4.68**	**1.20–15.69**	**0.013**	
<1 week	–	–	–		3.51	0.36–17.65	0.143	
>1 week	3.82	0.65–15.28	0.068		4.43	0.75–18.16	0.050	
Previous hospitalization	3.03	0.69–10.40	0.073		**3.55**	**0.79–12.59**	**0.049**	
<1 week	–	–	–		2.50	0.05–19.78	0.364	
>1 week	**4.67**	**1.03–16.61**	**0.023**		3.62	0.62–14.56	0.077	
Previous surgery	12.37	1.83–65.12	0.005		3.13	0.07–26.15	0.309	
Surgical or other wounds	3.25	0.86–10.38	0.041		**5.45**	**1.54–17.68**	**0.004**	
Bladder catheter	**7.47**	**1.59–28.27**	**0.006**		3.29	0.34–16.38	0.157	
Percutaneous gastrostomy	1.45	0.03–10.55	0.526		**15.19**	**3.53–58.75**	**0.000**	
Incontinence								
Urinary	1.46	0.47–5.33	0.617		–	–	–	
Faecal	1.38	0.46–3.98	0.619		**2.95**	**0.95–10.11**	**0.037**	
Care level								
1	**0.29**	**0.05–1.04**	**0.048**		–	–	–	
2	1.95	0.67–5.70	0.204		0.86	0.23–2.74	1	
3	1.28	0.30–4.24	0.754		**3.69**	**1.12–11.57**	**0.015**	
4	2.39	0.25–11.44	0.241		**7.16**	**1.52–27.08**	**0.007**	
Level of mobility								
Ambulant	**0.25**	**0.06–0.81**	**0.014**		**0.20**	**0.04–0.74**	**0.009**	
Wheelchair	**4.41**	**1.44–16.15**	**0.005**		1.62	0.52–5.08	0.433	
Bedridden	0.57	0.01–3.85	1.000		**4.99**	**1.28–16.82**	**0.010**	
Co-colonization								
Candida spp.	2.93	0.67–10.04	0.079		**4.83**	**1.24–16.23**	**0.012**	
Other gram negative species	3.12	0.83–9.92	0.047		1.08	0.12–4.96	1	

Three months after the initial survey, ESBL-producing Enterobacteriaceae were found again in 6 of the 16 initially colonized residents (37.5%).

Resistance phenotypes and genotypes of the six identified isolates were consistent with the corresponding strains detected at initial survey. The respective XbaI-macrorestriction patterns were identical or showed differences in only 1–3 bands in comparison to the initially isolated strains (Table 5).

Outcome

Reviewing medical records of all eligible residents enabled us to compare outcome parameters (occurrence of infections, inpatient admission, systemic antibiotic treatment or cases of death) during the past three months between colonized and non-colonized residents. At first follow-up, infections occurred in 21.7% of the residents without confirmed colonization ($n = 80$), while 42.4% of the colonized residents suffered from infections ($n = 15$; MRSA-colonized $n = 9$; ESBL-colonized $n = 5$; MRSA and ESBL-colonized $n = 1$), which reflects a significant cluster among the colonized residents (p-value 0.007). Distribution and spectrum of documented infections varied only slightly between the two groups (Fig. 1). Inpatient admission to an acute care hospital occurred in 14.1% ($n = 52$) of the non-colonized and 9.1% of the colonized residents ($n = 3$; MRSA-colonized $n = 1$; ESBL-colonized $n = 1$). Systemic antibiotic treatment was documented in 10.6% of the non-colonized ($n = 39$) and 18.2% of the colonized residents, reflecting a significant cluster in the group of colonized residents ($n = 6$; MRSA-colonized $n = 5$; ESBL-colonized $n = 1$; p-value 0.023). Fatalities occurred in 5.2% ($n = 19$) of the non-colonized and 3.0% of the colonized residents ($n = 1$; MRSA-colonized).

Table 4
Typing results and resistance phenotypes of isolated MRSA.

Nursing home	Resident	Initial sampling			First follow-up			Second follow-up		
		CC/ST	*Spa*-type	Resistance phenotype	CC/ST	*Spa*-type	Resistance phenotype	CC/ST	*Spa*-type	Resistance phenotype
201	P14	CC22	t032	PEN, OXA, CIP, MFL, OxaSu	No swab					
301	P40	CC5 ST225	t003	PEN, OXA, ERY, CLI, CIP, MFL, OxaSu	–	–	–	–	–	–
301	P04	(ESBL)	–	–	CC5 ST225	t002	PEN, OXA, GEN, ERY, CLI, CIP, MFL			
401	P25	ST97	t359	PEN, OXA, OxaSu	–	–	–	ST97	t359	PEN, OXA, OxaSu
401	P27	CC22	t032	PEN, OXA, CIP, MFL, OxaSu	CC22	t032	PEN, OXA, CIP, MFL, OxaSu	–	–	–
101	P27	CC22	t032	PEN, OXA, CIP, MFL, OxaSu	CC22	t032	PEN, OXA, ERY, CLI, CIP, MFL, OxaSu	–	–	–
101	P46	CC22	t492	PEN, OXA, ERY, CLI, CIP, MUP i, MFL, OxaSu	CC22	t492	PEN, OXA, CIP, MUP i, MFL, OxaSu	–	–	–
101	P53	CC22	t032	PEN, OXA, CIP, MFL, OxaSu	–	–	–	–	–	–
101	P74	CC22	t032	PEN, OXA, ERY, CLI, CIP, MFL, OxaSu	CC22	t032	PEN, OXA, ERY, CLI, CIP, MFL, OxaSu	–	–	–
101	P82	CC22	t032	PEN, OXA, ERY, CLI, CIP, MFL, OxaSu	CC22	t032	PEN, OXA, ERY, CLI, CIP, MFL, OxaSu	–	–	–
501	P04	CC5 ST225	t003	PEN, OXA, ERY, CLI, CIP, MFL, OxaSu	–	–	–	CC5 ST225	t003	PEN, OXA, ERY, CLI, CIP, MFL, OxaSu
501	P43	CC22	t646	PEN, OXA, ERY, CLI, CIP, MFL, OxaSu	–	–	–	CC22	t646	PEN, OXA, ERY, CLI, CIP, MFL, OxaSu
501	P52	CC22	t032	PEN, OXA, ERY, CLI, CIP, MFL, OxaSu	–	–	–	–	–	–
501	P15	CC5	t003	PEN, OXA, ERY, CLI, CIP, MFL, OxaSu	–	–	–	–	–	–
501	P77	ST22	t10442	PEN, OXA, ERY, CLI, CIP, MFL, OxaSu	–	–	–	ST22	t10442	PEN, OXA, ERY, CLI, CIP, MFL, OxaSu
402	P16	CC22	t032	PEN, OXA, CIP, MFL, OxaSu	CC22	t032	PEN, OXA, CIP, MFL, OxaSu	–	–	–
402	P25	CC22	t032	PEN, OXA, CIP, MFL, OxaSu	CC22	t032	PEN, OXA, CIP, MFL, OxaSu	CC22	t032	PEN, OXA, CIP, MFL, OxaSu
402	P29	CC22	t032	PEN, OXA, CIP, MFL, OxaSu	–	–	–	–	–	–
402	P35	CC5 ST225	t003	PEN, OXA, ERY, CLI, CIP, MUP, MFL, OxaSu	CC5 ST225	t003	PEN, OXA, ERY, CLI, CIP, MUP, MFL, OxaSu	CC5 ST225	t003	PEN, OXA, ERY, CLI, CIP, MUP, MFL, OxaSu
402	P41	CC22	t032	PEN, OXA, CIP, MFL, OxaSu	CC22	t032	PEN, OXA, CIP, MFL, OxaSu	–	–	–

No entry at follow-ups: no colonization; no swab: resident refused further participation, died or moved out; CC: clonal complex; ST: sequence type.

Table 5
Resistance phenotypes and genotypic characteristics of ESBL-producing Enterobacteriaceae.

Nursing home	Resident	Initial sampling						First follow-up (after 3 months)						Second follow-up (after six months)					
		Species	ESBL	Other β-lactamases		*E. coli* phylotype	PFGE-type	Species	ESBL	Other β-lactamases		*E. coli* phylotype	PFGE-type	Species	ESBL	Other β-lactamases		*E. coli* phylotype	PFGE-type
301	P01	E. coli	CTX-M-1	–	–	B1	1	E. coli	CTX-M-1	–	–	B1	1a	E. coli	CTX-M-1	–	–	B1	1b
301	P04	E. coli	CTX-M-1	TEM-1	–	B1	2	–	–	–	–			E. coli	CTX-M-14	TEM-1	–	B2	3
301	P49	E. coli	CTX-M-1	–	–	A	14	–	–	–	–			–	–	–	–		
401	P16	K. pneumonia	CTX-M-15	TEM-1	SHV-1		4	K. pneumoniae	CTX-M-15	TEM-1	SHV-1		4a	No swab					
101	P32	K. pneumoniae	SHV-12	TEM-1	SHV-1		8	K. pneumoniae	SHV-12		SHV-1		8a	–	–	–	–		
101	P37	K. pneumoniae	SHV-12	–	SHV-1		9	–	–	–	–			–	–	–	–		
101	P51	E. coli	CTX-M-1	TEM-1	–	A	15	–	–	–	–			E. coli	CTX-M-1			B1	16
601	P31	E. coli	CTX-M-15	–	–	B2	7	–	–	–	–			E. coli	CTX-M-15	–	–	B2	7a
601	P41	E. coli	CTX-M-15	TEM-1	–	A	11	–	–	–	–			–	–	–	–		
601	P48	E. coli	CTX-M-15	TEM-1	–	B2	10	E. coli	CTX-M-15	TEM-1	–	B2	10a	E. coli	CTX-M-15	TEM-1	–	B2	10a
601	P52	E. coli	CTX-M-1	–	–	B1	17	No swab						No swab					
501	P29	E. coli	CTX-M-1	–	–	B2	6	E. coli	CTX-M-1	–	–	B2	6a	No swab					
501	P52	E. coli	CTX-M-1	–	–	B1	18	–	–	–	–			E. coli	CTX-M-1	–	–	B1	18
501	P79	K. pneumoniae	CTX-M-15	TEM-1	SHV-1		19	–	–	–	–			K. pneumonia	CTX-M-15	TEM-1	SHV-1		19
402	P25	E. coli	CTX-M-14	–	–	B2	5	E. coli	CTX-M-14	–	–	B2	5a	E. coli	CTX-M-14	–	–	B2	5b
402	P37	E. coli	CTX-M-15	–	–	B2	10b	–	–	–	–			–	–	–	–		

No entry at follow-ups: no colonization; no swab: resident refused further participation, died or moved out.

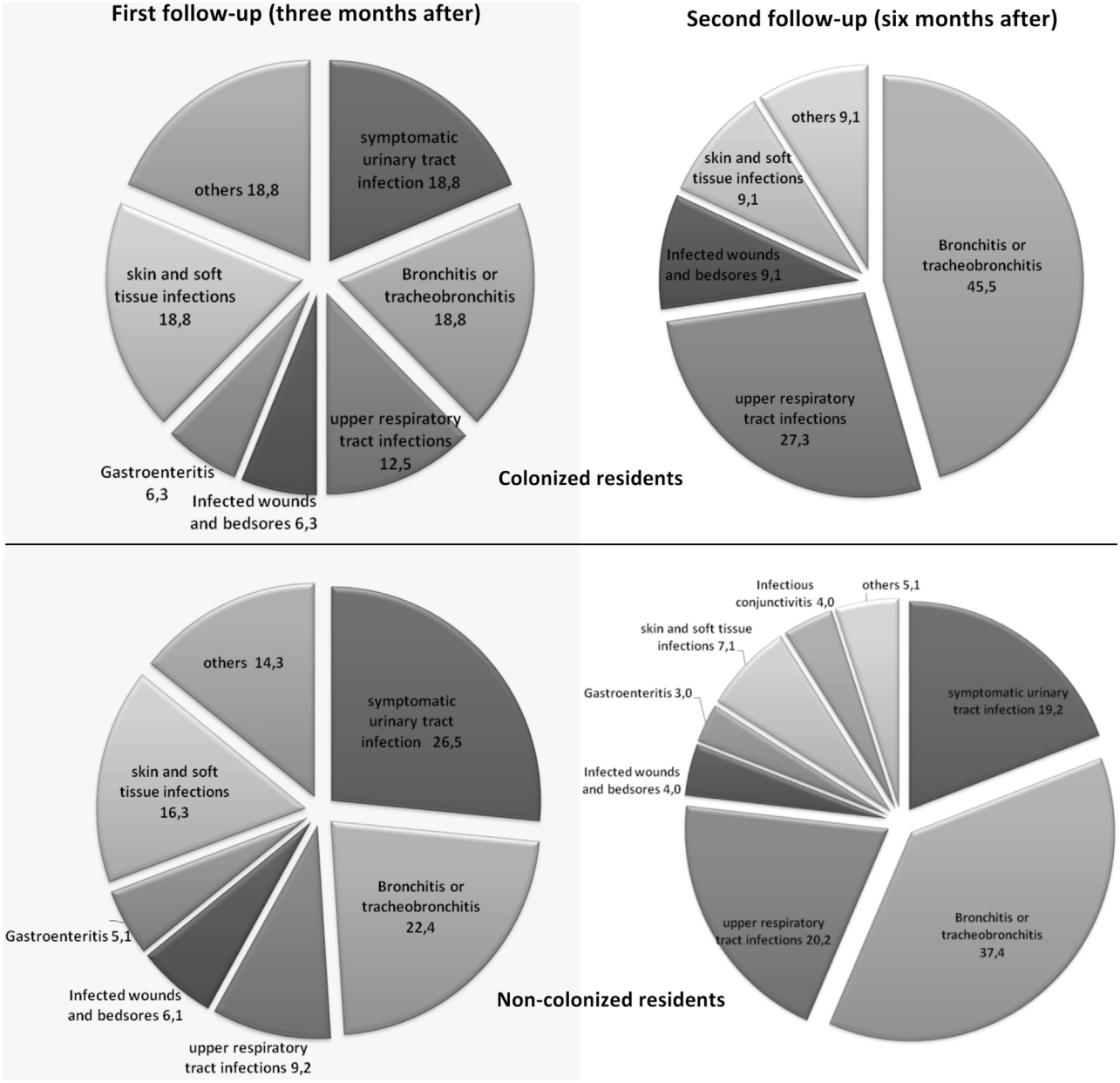

Fig. 1. Percentages of documented infections in residents colonized with MRSA and/or ESBL-producing bacteria and non-colonized residents at first and second follow-up survey.

Second follow-up (6 months after)

MRSA and ESBL-colonization

At the second follow-up screening, six of the initially MRSA-colonized residents were shown to be colonized with MRSA (31.5%). Over the past three months between both follow-ups, genetic affiliations remained constant for each strain (Table 4). MRSA decolonization treatment after the first follow-up was performed for seven residents, yet again written documentation of actually performed procedures was poor or not done at all.

Six months after the initial survey, ESBL-producing Enterobacteriaceae were found in 8 (50%) of the initially colonized residents, three of them (all *E. coli*) were also present at the first follow-up screening, each of the three was colonized with the respective initially strain (Table 5).

Outcome

Infections within the past three months after the first follow-up were documented in 25.6% of the non-colonized residents ($n = 89$) and 40.6% of the residents colonized by a multidrug-resistant pathogen ($n = 13$; MRSA-colonized $n = 8$; ESBL-colonized $n = 3$; ESBL- and MRSA colonized $n = 2$; p-value = 0.054). As observed in the first follow-up the spectrum and proportion of occurred infections showed only few differences in both groups, with urinary and respiratory tract infections being the most prevalent types of infections in both groups (Fig. 1).

Inpatient admission to an acute care hospital was documented in 15.8% of the non-colonized residents ($n = 55$) and in 9.4% of the colonized residents ($n = 3$; MRSA-colonized $n = 2$; ESBL-colonized $n = 1$). Antibiotics prescribed for systemic use were documented in 14.7% of the non-colonized residents ($n = 51$) and 18.7% of the

colonized residents ($n = 6$; MRSA-colonized $n = 5$; ESBL-colonized $n = 1$). Cases of death during the past three months occurred in 6.6% of the non-colonized residents ($n = 23$) and 12.5% of the colonized-residents ($n = 4$; MRSA-colonized $n = 2$; ESBL-colonized $n = 2$). Statistically significant clusters were not observed at second follow-up. Multivariate analysis of risk factors for infection in the study population did not reveal significant clusters in terms of colonization with multiresistant bacteria. The presence of urinary catheters and dementia were the only factors of importance, nonetheless without reaching the level of significance ($p = 0.1$).

Discussion

Our multicenter survey in seven elderly care facilities revealed that the prevalence of inguinal skin colonization with multidrug-resistant bacteria was 8.2%, including 4.7% MRSA-carriage and 4.0% carriage of ESBL-producing Enterobacteriaceae.

In Germany, Arvand et al. (2013) isolated 9.6% ESBL-producing Enterobacteriaceae from faecal samples of 240 residents in 11 nursing homes with prevalence rates ranging from 0% to 30%. Another recent German survey screened 178 residents in eight nursing homes for multidrug resistant bacteria and found an overall prevalence of 11.2% ESBL-producing bacteria from rectal swabs (Gruber et al., 2013). Prevalence rates between 32% and 64% from rectal swabs or faecal samples of nursing home residents were reported from the USA, Italy and United Kingdom and raised concern about potential clinical implications and appropriate precaution measures in long term care facilities (March et al., 2009; Pop-Vicas et al., 2008; Rooney et al., 2009; Trick et al., 2001). However, Lautenbach et al. (2012) detected a comparable low prevalence of 3.4% ESBL-producing bacteria from perirectal swabs in a multicenter study in three long term care facilities in the USA and stressed a significant interfacility range out (0.8–10.7%). A large national survey concerning the epidemiology of multidrug-resistant bacteria was recently conducted in 60 Belgian nursing homes and also revealed a rather low prevalence of ESBL-producing bacteria (6.2%; range: 0–20%) although a broader set of sample sites including rectal swabs was comprised.

The comparatively low prevalence of asymptomatic carriage of both MRSA and ESBL-producing bacteria in our study might be due to the fact that we focused on colonization of the inguinal skin and did not include nasal or rectal sampling. Buehlmann et al. (2010) revealed the inguinal skin as an important site for ESBL-colonization and also an Italian study in long term care facilities observed a frequent colonization of the inguinal skin with both, MRSA and ESBL-producing Enterobacteriaceae (March et al., 2009). March et al. (2009) found ESBL-positive inguinal swabs in 82% of the ESBL-positive residents, presuming a very frequent inguinal colonization of ESBL-producing bacteria. It appears plausible that multidrug-resistant bacteria, detectable without prior enrichment from the inguinal skin, might have a higher impact on transmission compared to the detection in stool samples after enrichment, which led us to the decision to focus on that site.

Differences in staffing level, implementation of standard precaution measures and also the individual resident populations and antibiotic use patterns of general practitioners in the facilities were discussed as possible explanations for wide interfacility ranges of prevalence rates. Indeed there might be a variety of complex individual factors within the facilities that are hard to discern during epidemiological surveys.

In accordance with other studies, the predominant ESBL-producing species in our study were *E. coli* (75%) and *K. pneumoniae* (25%) harbouring primarily $bla_{\text{CTX-M-15}}$ and $bla_{\text{CTX-M-1}}$ genes (Gruber et al., 2013; Jans et al., 2013) Co-resistance against Ciprofloxacin occurred in the CTX-M-15 harbouring isolates, as expected. Both CTX-M-15 and CTXM-1 are widely disseminated and frequently found in isolates causing nosocomial infections not only in Germany (Pfeifer et al., 2010).

Considering frequent hospital admissions of long term care residents a prospective cohort study in Belgium, Schoevaerdts et al. (2012) screened patients newly admitted to a geriatric unit for the occurrence of multidrug-resistant bacteria. They found 14% of the patients admitted from nursing homes being colonized with ESBL-producing Enterobacteriaceae, also mainly consisting of CTX-M group 1 harbouring isolates. Interestingly, ESBL prevalence at admission did not differ substantially between patients admitted from home and those admitted from nursing homes. Gruber et al., however, detected a lower prevalence of ESBL in patients of ambulant care facilities (4.7%) compared to residents of nursing homes (9%). These findings imply that individual risk factors rather than the accommodation in care facilities might contribute to the asymptomatic carriage of ESBL-producing bacteria. Nonetheless, comparing (admission) prevalence rates of different health care sections, it is noteworthy that a recent German study revealed an intestinal colonization with ESBL-producing bacteria in 6.5% of the general community, independently of age and gender (Valenza et al., 2014).

Strain typing of ESBL-producing bacteria in the present study did not indicate major cross-transmission events or cluster effects between residents of the respective facilities. Only in two residents from two different facilities an identical CTX-M-15-positive *E. coli* strain (phylogroup B2) was found. It is known that specific clonal lineages of *E. coli* have been spread worldwide, e.g. the CTX-M-15-positive *E. coli* O25b:H4-ST131-B2 but the plasmid transfer in various strains may also contribute to the successful spread of ESBLs in *E. coli* (Arvand et al., 2013; Woodford et al., 2011). Our analyses show that colonized residents mainly harbour “their own” ESBL-producing strains, which underwent only slight genetic alterations (1–3 bands in macrorestriction pattern) during the study period. Jans et al. (2013) also observed less important cluster effects for ESBL-producing *E. coli* compared to other resistant bacteria in the large Belgian multicenter study. This suggests that de novo acquisition of resistance rather than direct cross-transmission between residents occurred, what emphasizes the importance of individual risk factors like previous antibiotic exposure for asymptomatic ESBL-carriage.

Previous antibiotic exposure, in particular to fluoroquinolones and third generation cephalosporins, is frequently described to be associated with carriage of ESBL-producing bacteria in nursing home residents (Mendelson et al., 2005; Sandoval et al., 2004). This appears to be a dose related process since it was shown that the duration of exposure plays an important role in the selection of resistant strains (Rooney et al., 2009; Tinelli et al., 2012). Hence, de novo acquisition of resistance, potentially supported by selective pressure imposed by the use of broad-spectrum antibiotics, may be of particular importance with respect to the epidemiology of ESBL-producing bacteria in long term care facilities.

Both follow-up surveys (three and six months after initial sampling) revealed a large proportion of residents with persistent ESBL-carriage (37.5% at first follow-up and 50% at second follow-up, Table 5). Acquisition of new strains along the study period occurred only in two patients. A long duration of gastrointestinal colonization with ESBL-producing bacteria is frequently reported; for example a very recent study investigated the gastrointestinal ESBL-colonization of hospital patients after discharge and found a median time to clearance of 6.6 months, ranging between 3.4 and 13.4 months (Birgand et al., 2013). O’Fallon et al. (2009a) investigated serial surveillance cultures from nursing home residents for multidrug-resistant gram-negative bacteria and found a median duration of colonization of 144 days, ranging between 41 and 349 days. Similar results were reported from Maslow et al. (2005) for

gastrointestinal carriage of fluoroquinolone resistant *E. coli* in a long term care facility, where the median time for clearance was five months, ranging between two and ten months. As a consequence, healthcare workers should be aware of a possibly long persistence of multidrug-resistant bacteria even on the inguinal skin and the according requirements for accurate standard precaution measures and pay special attention to residents presenting risk factors for colonization or infection with multi-resistant bacteria.

Compared to recent prevalence surveys in long term care facilities, we detected a lower prevalence of asymptomatic MRSA carriage, but this may be attributed to the sampling of inguinal swabs only. Recently, Gruber et al. found 9% of the nursing home residents colonized with MRSA, including swabs from nose, throat, rectum and wounds. The large national survey from Belgium revealed a MRSA prevalence of 12.2% from similar sampling sets (Jans et al., 2013). Another large multicenter survey in 32 German nursing homes showed an overall prevalence of 7.6% from nasal and wound swabs (Pfingsten-Würzburg et al., 2011), while a very high MRSA prevalence (38.7%) was reported from Italian nursing homes and also from a very large multicenter survey in Great Britain (22%) (Barr et al., 2007). Schoevaerdts et al. (2012) found 19% of patients admitted from nursing homes to a geriatric hospital unit colonized with MRSA, which is in significant contrast to patients that were admitted from home (5%). Gruber et al. also revealed a much higher MRSA prevalence in nursing home residents (11.2%) compared to those of ambulant care facilities (4.7%). These facts imply that for the epidemiology of MRSA, nursing homes may indeed play a very important role. It has to be mentioned however that defined nursing home characteristics i.e. a higher proportion of residents with indwelling devices or chronic illness do not only influence the MRSA prevalence but also the risk of transmission and may explain the substantial interfacility variety of MRSA burden (Murphy et al., 2012).

Molecular typing of the isolated MRSA strains in our study revealed the vast majority being clonally related to spa type t032, CC22 ("Barnim" epidemic strain) which is a frequently occurring lineage in Germany and the most prevalent lineage in German hospitals (Grundmann et al., 2010; Schaumburg et al., 2012). Beside t032, we also found strains related to the spa type t003 CC5 lineage ("Rhein-Hessen" subtype epidemic strain), which is another wide spread lineage in German hospitals. These results are in accordance to those of the more recent multicenter studies in Germany, were the two lineages were also predominant (Gruber et al., 2013; Pfingsten-Würzburg et al., 2011).

In addition to the common lineages t032 and t003 we found one strain of a quite rare lineage (ST97, t359) that is described from cattle and swine and appears just very sporadically in humans. Two further isolated MRSA of clonal lineage CC22 belonged to more sporadic spa-types t646 and t492, which were described in Germany before (spa server). In addition, we found one isolate of ST22 harbouring a new spa type (t10442) not described so far. Isolates of the Barnim and Rhein-Hessen epidemic type as well as the sporadic t646, t492 and the new t10442 showed co-resistance to ciprofloxacin and erythromycin/clindamycin. Furthermore antimicrobial resistance testing revealed two of the initially 19 isolated MRSA (10.5%) being mupirocin non-susceptible (one isolate resistant and the other one intermediate), reflecting the upcoming rise of mupirocin-resistant MRSA in hospitalized patients worldwide (McDanel et al., 2013; Rossney and O'Connell, 2008).

Inconsistent decolonization regimen might be the cause for the observed persistence rates of MRSA of 47.3% at three months and 31.5% at six months after initial detection in our study. Extranasal MRSA-colonization was shown to be frequent, especially in nursing home residents with indwelling devices (Mody et al., 2008). Furthermore, different studies observed that faecal carriage of MRSA is common also in residents of nursing homes (Klotz, Zimmermann et al., 2005) which accounts for the frequent isolation of MRSA from inguinal swabs in incontinent residents. March et al. (2009) found in 67% of their MRSA-colonized LTCF-residents MRSA-positive cultures from inguinal swabs and in 86% positive cultures from the combination of rectal and inguinal swabs. As assumed by Klotz et al., faecal carriage of MRSA might play an important role in re-colonization after successful decolonization, which might also be a possible reason for persistent inguinal MRSA carriage in our study. In a cohort study that investigated MRSA positive hospital patients over a time period of more than three years, long-term persistence of MRSA in patients who were re-admitted was described as a very frequent phenomenon and MRSA carriage at multiple anatomic sites appeared to be predictive for a prolonged persistence (Mattner et al., 2010). Thus, MRSA long-term persistence and multiple site colonization including a frequent intestinal colonization of residents should be considered also in nursing homes and accounted for in appropriate facility-wide decolonization protocols.

Risk factors associated with MRSA carriage were previous hospitalization for more than one week, urinary catheter and a known history of multidrug-resistant bacteria colonization, which is also in accordance with findings of other studies (Gruber et al., 2013; Jans et al., 2013; Mody et al., 2007; Wang et al., 2012). We found the presence of wounds and skin lesions as well as a previously known carriage of multidrug resistant bacteria as independent risk factors for MRSA carriage and indeed those factors seem to contribute substantially to the burden of MRSA in nursing homes and should be considered in terms of precaution measures (Gruber et al., 2013; Jans et al., 2013). A previously known MRSA carriage might be an indication for an individual disposition of the skin and mucosa for colonization with *S. aureus.* In addition to further risk factors like fluorquinolone exposure or hospitalization this might result in MRSA colonization. Thus, facilities should pay attention to those residents in order to prevent further risk factors and avoid subsequent infections.

Independent risk factors as results of logistic regression analysis for asymptomatic colonization with both, MRSA and/or ESBL-producing bacteria in our study were a high level of care and the presence of chronic wounds. Furthermore, univariate analysis revealed previous antibiotic treatment, wounds, percutaneous gastrostomy, a high level of care and immobility as risk factors for ESBL-carriage. A low functional status or immobility was found to be a strong predictor for asymptomatic carriage of multiresistant bacteria in other studies in nursing homes. Physical disability and a low level of mobility are identified as risk factors in multiple studies (Gruber et al., 2013; March et al., 2009; Pop-Vicas et al., 2008; Schoevaerdts et al., 2012). Jans et al. (2013) assumed that a low level of autonomy is associated with a higher prevalence of potential risk factors for acquisition of multidrug-resistant bacteria, a higher risk of cross transmission and also of antibiotic exposure and proposed to assess the functional status of nursing home residents in the medical evaluation of patients at risk for colonization or infection with multidrug-resistant bacteria.

In contrast to MSRA and ESBL-producing bacteria, an asymptomatic carriage of vancomycin-resistant enterococci (VRE) was not detected in our study. This is consistent to recently published findings, were VRE occurred either very rarely or not at all in residents of European nursing homes (Gruber et al., 2013; Jans et al., 2013; March et al., 2009; Schoevaerdts et al., 2012). It was supposed that the infrequent use of glycopeptide antimicrobials in nursing homes reduces the antibiotic selection pressure and attributes to the low prevalence. However, international data from the past years also reported higher prevalences emphasizing the remarkable environmental spread of VRE in nursing homes (Elizaga et al., 2002). Hence, the epidemiology of VRE in nursing homes seems to be complex and risk factors on individual and facility level need to be further investigated to assess its actual importance in this setting.

In the present study we found a large amount of residents (12%) with inguinal colonization of other gram-negative bacteria displaying cefpodoxim-resistance (*M. morgannii*, *K. oxytoca*, *Pseudomonas* spp., *Acinetobacter* spp.) as well as *Candida* spp. (9.5%). Moreover, we found a significant proportion of co-colonization with these bacteria, *Candida* spp., MRSA and ESBL-producers in our study population. The resistance to 3rd generation cephalosporins in other identified *M. morgannii* and *K. oxytoca*, was not due to ESBL-presence but highly probable due to production of intrinsic AmpC-type beta-lactamases (DHA-1 in *M. morgannii*) and OXY-type beta-lactamases in *K. oxytoca*, respectively. *P. aeruginosa* with remarkable resistance to ceftazidime and ciprofloxacin were found in several patients. An inguinal skin colonization of these bacteria might be an indicator for suboptimal skin care, especially in incontinent and immobile residents. *Pseudomonas* spp. was described as a very frequent causative agent in skin and soft tissue infections in the elderly (Kish et al., 2010) and *Candida* spp. is known to be involved in the development of incontinence associated dermatitis in the elderly (Beeckman et al., 2009). Since a healthy and intact skin presents a physical barrier against invasion of microorganisms, an adequate concept of skincare for the elderly is crucial to keep the integrity of the epidermis and avoid bacterial overgrowth and infections.

Comparing occurrence of infections, hospital admissions and use of antibiotics of colonized versus non-colonized residents we found statistically significant associations for infections and antibiotic prescriptions among the colonized residents only at the first follow-up. The spectrum and proportion of documented infections did not differ substantially between both groups. No association was found between colonization with multidrug-resistant bacteria and frequency of hospital admission or fatalities. However, statistical analysis was limited due to the low number of colonized residents at the two follow-ups. In a prospective cohort study of hospitalized elderly patients, it was recently shown that new acquired infections, mortality or length of stay in the hospital did not differ between patients colonized with MRSA or ESBL and non-colonized patients (Schoevaerdts et al., 2012). Considering the observed attributes of inguinal colonized residents in our study and the spectrum of infections in both groups, it appears that not the colonization with multidrug-resistant bacteria intrinsically leads to a higher prevalence of infections. It is rather reasonable that a particular population of residents in nursing homes is vulnerable to both, colonization with multidrug resistant bacteria as well as healthcare associated infections.

Our study has several limitations that need to be mentioned. First, diagnostic sensitivity of inguinal screening for ESBL and MRSA is not as high as rectal and nasal/throat screening methods (March et al., 2009; Mertz et al., 2007), which might have an influence on comparability of our prevalence data with other screening studies. An underestimation of overall asymptomatic colonization with multidrug-resistant bacteria can be assumed and should be considered when interpreting the results. On the other hand, it should also be considered that factors like immobility as well as incontinence may lead to a higher inguinal detection rate of bacterial pathogens.

Another aspect is that the local recruitment of voluntarily participating elderly care facilities in an urban region affects generalization of our data. Furthermore, the sample size (seven nursing homes, 402 eligible residents) might not be sufficient for statistically reliable analysis of outcome measurements. The microbiological follow-up screenings were performed only in residents who were found to be colonized with one of the target microorganisms at the initial survey. We were not able to assess whether residents of the "non-colonized cohort" potentially changed their status and got colonized among the whole study period. This bias can possibly lead to a further underestimation of outcome measurements in colonized residents.

In spite of these limitations, we found that the inguinal skin of elderly nursing home residents is frequently colonized with MRSA, ESBL-producing bacteria and colonization of these organisms tends to be persistent over several months. Healthcare workers and nurses need to bear in mind that a large proportion of residents tend to be colonized without signs or symptoms of infections and standard precautions and measures of hand hygiene in the field of long term care should consider these facts. Furthermore, preventive infection control concepts in the elderly should attentively integrate strategies for skin care, especially for incontinent residents in order to maintain an intact physical barrier. Decolonization schemes for MRSA colonized residents should be reviewed and performed according to appropriate protocols, also considering frequent extranasal MRSA colonization.

In conclusion, we assume that a perceptible population displaying a certain pattern of risk factors (i.e. frail residents with a high level of care, immobility and dependency) is at risk for inguinal colonization with multidrug-resistant bacteria. While it is indistinct if colonization itself or rather the composition of risk factors leads to a poorer outcome, those residents also tend to develop healthcare associated infections more frequently. Residents with poor functional status and increased requirements of care are more likely to have a higher frequency of infections requiring antibiotic treatment, which can subsequently lead to the acquisition of resistant organisms. Those residents need to be cared for with special attention to prevent infections and bacterial cross-transmission.

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