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Surveillance of bacterial colonisation on contact surfaces in different medical wards

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This study was conducted to determine the bacterial colonization of some bacterial groups, including extended–spectrum β-lactamase (ESBLs) producers and methicillin-resistant *Staphylococcus aureus* (MRSA), on surfaces of the equipment and instruments in patient rooms and other workspaces in three different medical wards. The number of microorganisms on swabs was determined with the colony count method on selective microbiological mediums. The aerobic mesophylic microorganisms were found in 73.5 % out of 102 samples, with the average and maximum values of 2.6×10^2 and 4.6×10^3 colony forming units (CFU) 100 cm^{-2}, respectively. Members of the family *Enterobacteriaceae*, coagulase positive staphylococci, coagulase-negative staphylococci, and enterococci were detected in 23.4, 31.4, 53.2, and 2.9 % of samples, respectively. The differences in bacterial counts on the surfaces of the psychiatric, oncology, and paediatric wards were statistically significant ($P<0.001$). About 40 % out of 19 isolates from the family *Enterobacteriaceae* showed multiple resistance to three or more different groups of tested antibiotics, while ESBL was confirmed for only one strain. Staphylococci isolates were mostly resistant to penicillin. MRSA was confirmed in 5.2 % of the tested *S. aureus* isolates. Greater attention should be paid to cleaning and the appropriate choice of disinfectants, especially in the psychiatric ward. Employees should be informed about the prevention of the spreading of nosocomial infections. Routine application of rapid methods for hygiene control of surfaces is highly recommended.

KEY WORDS: *antibiotic susceptibility; bacterial contamination; hospitals; infections*

Healthcare-acquired infections (HAIs) have been recognised as a critical problem affecting the quality of healthcare (1). The increasing emergence and spread of multiresistant bacteria in hospitals is a serious concern and continues to challenge infection control and hospital epidemiology practice worldwide (2). Gram–positive *Clostridium difficile*, methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci (VRE), and multidrug-resistant Gram-negative rods such as extended spectrum β-lactamase (ESBL)-producing *Enterobacteriaceae,* particularly *Klebsiella pneumoniae, Escherichia coli, Enterobacter* spp.*, Citrobacter* spp.*,* and *Serratia* spp.*,* including *Acinetobacter baumanii*, share the ability to be shed from infected or colonised patients. One critical aspect of bacterial transfer is the ability of the microorganism to survive on various hospital surfaces for extended periods of time and the fact that it is difficult to eradicate it by cleaning and disinfection (3, 4). Pathogens contaminating environmental surfaces can spread to patients by direct contact with the surface or indirectly, typically on the hands of healthcare workers (5). Although hand hygiene is important in order to minimise the impact of this transfer, cleaning and disinfecting environmental surfaces appropriately is fundamental for reducing their potential contribution to the incidence of healthcare-associated infections (6).

In this study, we evaluated the level of contamination of surfaces in patient rooms and medical equipment in various medical wards with some main bacterial groups. We investigated the antibiotic resistance of isolated *Enterobacteriaceae* and staphylococci, including the determination of ESBL and MRSA bacterial strains.

MATERIALS AND METHODS

Sampling

The number and presence of individual groups of microorganisms on 17 selected surfaces of the equipment and supplies in patient rooms as well as in other clinical facilities in oncology, paediatric, and psychiatric medical wards was determined.

The samples were collected twice, in February 2014 and September 2014, to avoid the influence of daily and seasonal fluctuations in cleaning procedures and the number of microorganisms on the results. The samples were taken in the afternoons after the lunches have been served. One hundred and two (102) swabs with 10 mL of saline solution taken on 100 cm^2 of selected surfaces were collected according to the international standard (7). The collecting

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points listed in Table 2 were identical in all three medical wards.

The evaluation of bacterial count and identification of isolates

The aerobic colony count (ACC), the number of *Enterobacteriaceae* (EB), coagulase-positive staphylococci (CPS), coagulase-negative staphylococci (CNS), and enterococci (EC) were determined in collected swabs using the standard plate count method. One mL of the bacterial suspension was transferred from the swab into a petri dish, and an appropriate medium was poured over it (7, 8).

The presence and number of aerobic mesophylic microorganisms were evaluated as ACC by counting the growing colonies on PCA agar after incubation under aerobic conditions at 30 °C for 72 hours (Merck, Germany) (8).

The colonies of presumptive mannitol-positive staphylococci and mannitol-negative staphylococci were differentiated and counted on the Mannitol Salt Phenol Agar (Merck, Germany). After 48 h incubation at 37 °C, red or colourless colonies were produced by mannitol negative and presumptive coagulase-negative staphylococci. Small to large colonies with yellow zones were confirmed by inoculation on Baird Parker medium, supplemented with Rabbit Plasma Fibrinogene (RPF) (Biolife, Italy). Black colonies surrounded by white precipitation zones were presumed to be CPS (9, 10). Strain identification was performed using the detection of haemolytic activity, catalase and oxidase test, and biochemical characterisation with API Staph (bioMerieux, France) according to the manufacturer's instruction.

The enumeration of members of *Enterobacteriaceae* was carried out on MacConkey agar (Merck, Germany) after 24 h incubation at 35 °C according to the standard (10, 11). Typical red (lactose-fermenters), colourless or pink (non-lactose fermenters) colonies were confirmed with Gram-stain microscopy, catalase and oxidase production, and biochemical tests API 10S (bioMerieux, France).

The presence and number of colonies of the genus *Enterococcus* were detected on KF Streptococcus Agar Base with TTC supplement (Merck, Germany) after 24 h incubation at 37 °C and confirmed on Bile Esculin Azide agar (Merck, Germany) (10).

The results were expressed as the number of colony-forming units (CFU) per 100 cm^2 of sampled surfaces.

Antibiotic susceptibility testing

The antimicrobial susceptibility of 13 isolates from fam. *Enterobacteriaceae* and 46 isolates of staphylococci, obtained from the chosen surfaces were determined by using the Kirby-Bauer disc diffusion method. The control strains used to validate the antimicrobial susceptibility testing were *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922, and *K. pneumoniae* ATCC 700603 (12).

The antibiotic discs for detecting the susceptibility of enterobacteria were cephalexin (CN-30 μg), tobramycin (NN-10 μg), nalidixic acid (NA-30 μg), ciprofloxacin (CIP-5 μg), and trimethoprime (TMP-5 μg). The ESBL strains were confirmed using a combined disc diffusion test using cefotaxime (CTX-30), cefotaxime with clavulanate (CTX-30/CA-10), ceftazidime (CAZ-30), ceftazidime with clavulanate (CAZ-30/CA-10), cefpodoxime (CPD-10 μg), and cefpodoxime with clavulanate (CPD-10 μg /CA-1 μg) (Mast Diagnostics, UK). MBLs were detected by combined disc test with imipenem and meropenem discs (10 μg each) (Mast Diagnostics, UK) alone and with 10 μL of 0.5 mol L^{-1} EDTA (pH=8). An augmentation of the inhibition zone of ≥5 mm in the presence of clavulanate or EDTA pointed to the production of ESBL or MBLs (carbapenemases group), respectively (12).

The antibiotic discs for detecting the susceptibility of staphylococci were penicillin (P-10 EU), erythromycin (E-15), ciprofloxacin (CIP-5 μg), clindamycin (CC-2 μg), and kanamycin (K-30 μg) (BBL Becton Dickinson, UK). Methicillin and vancomycin resistance was determined using the oxacillin and vancomycin E-test (AB Biodisk, Solna, Sweden), respectively (12).

Production of inducible β-lactamases

The presence of induced β-lactamases at the strains *Staphylococcus* and *Enterobacteriaceae* after exposure to penicillin and cefotaxime discs (BBL Becton Dickinson, UK) was also determined using a Cefinase (Cef-F) test with chromogenic cephalosporin (bioMerieux, France) (12).

Genotypic detection of genes for β-lactamases at enterobacteria isolates

The overnight cultures in BHI broth were centrifuged at 15,000 × *g* for 15 min. The supernatant was eliminated, and the pellet was resuspended in molecular biology-grade water and centrifuged at 15,000 × *g* for 10 min. After elimination of the supernatant, the pellet was resuspended in 40 μL of molecular biology-grade water, subjected to boiling at 100 °C in a water bath for 10 min, cooled on ice, and centrifuged at 15,000 × *g* for 10 s. Aliquots of 2 μL of template DNA were used for PCR (13). The multiplex PCR with specific primers for bla_{CTX-M} groups 1, 2, 8, 9, and 25 was used. For detecting the variants from the families of bla_{VIM}, bla_{IMP}, as well as the variants bla_{GIM}, bla_{SPM-1}, and bla_{SIM-1}, multiplex primers bla_{MBL} were used (14, 15). The primers and cycling conditions are shown in Table 1.

Statistical analysis

The programmes IBM SPSS Statistics 17 (2012) and Excel 2006 were used for statistical analysis of data. ANOVA was used for calculating the mean values, maximum and minimum values, standard deviations, while a t-test showed the statistical significance of differences between the mean values of microorganisms in medical

Table 1 *Oligonucleotide primers used for detecting β-lactamase genes*

Target sequence	Nucleotide sequence (5'→3')	Orientation	Amplicon's expected size (bp)	PCR conditions	Reference
bla$_{CTX-M1}$	AAA AAT CAC TGC GCC AGT TC	F	415	94 °C /5 min; 30 cycles 94 °C/25 s, 52 °C/40 s, 72 °C/50 s; 72 °C/6 min (15)	(15)
	AGC TTA TTC ATC GCC ACG TT	R			
bla$_{CTX-M2}$	CGA CGC TAC CCC TGC TAT T	F	552		
	CCA GCG TCA GAT TTT TCA GG	R			
bla$_{CTX-M8}$	TCG CGT TAA GCG GAT GAT GC	F	666		
	AAC CCA CGA TGT GGG TAG C	R			
bla$_{CTX-M9}$	CAA AGA GAG TGC AAC GGA TG	F	205		
	ATT GGA AAG CGT TCA TCA CC	R			
bla$_{CTX-M25}$	GCA CGA TGA CAT TCG GG	F	327		
	AAC CCA CGA TGT GGG TAG C	R			
bla$_{IMP}$	GGA ATA GAG TGG CTT AAT TCT C	F	188		(14, 15)
	CCA AAC CAC TAC GTT ATC T	R			
bla$_{VIM}$	GAT GGT GTT TGG TCG CAT A	F	390		
	CGA ATG CGC AGC ACC AG	R			
bla$_{GIM}$	TCG ACA CAC CTT GGT CTG AA	F	477		
	AAC TTC CAA CTT TGC CAT GC	R			
bla$_{SPM}$	AAA ATC TGG GTA CGC AAA CG	F	271		
	ACA TTA TCC GCT GGA ACA GG	R			
bla$_{SIM}$	TAC AAG GGA TTC GGC ARC G	F	570		
	TAA TGG CCT GTT CCC ATG TG	R			

wards and between seasons. The rate of statistical significance was determined with value $P \leq 0.05$.

RESULTS

The level of surface contamination in medical wards

ACC and CNS were present in 73.5 % and 53.2 % out of 102 collected swabs, respectively. *Enterobacteriaceae* were present in only 26.6 % of samples, mostly on surfaces of the psychiatric ward (Figure 1). The number of ACC exceeded the recommended values ≤ 250 CFU 100 cm^{-2} for surfaces which were in contact with hands in patient rooms (17) in the psychiatric, paediatric, and oncology wards in 38.2 %, 5.9 %, and 0 % of samples, respectively.

The most frequently contaminated surfaces were those of telephone headsets, night tables, wheelchair handles, serving trays, bedside frames, and water taps (Table 2). The highest number of ACC with the average and maximum values of 2.6×10^2 and 4.6×10^3 CFU 100 cm^{-2}, respectively, was followed by the number of CNS and CPS (Figure 2). The contamination of surfaces was the highest in the psychiatric ward, while the lowest number of positive samples, as well as the lowest number of microorganisms,

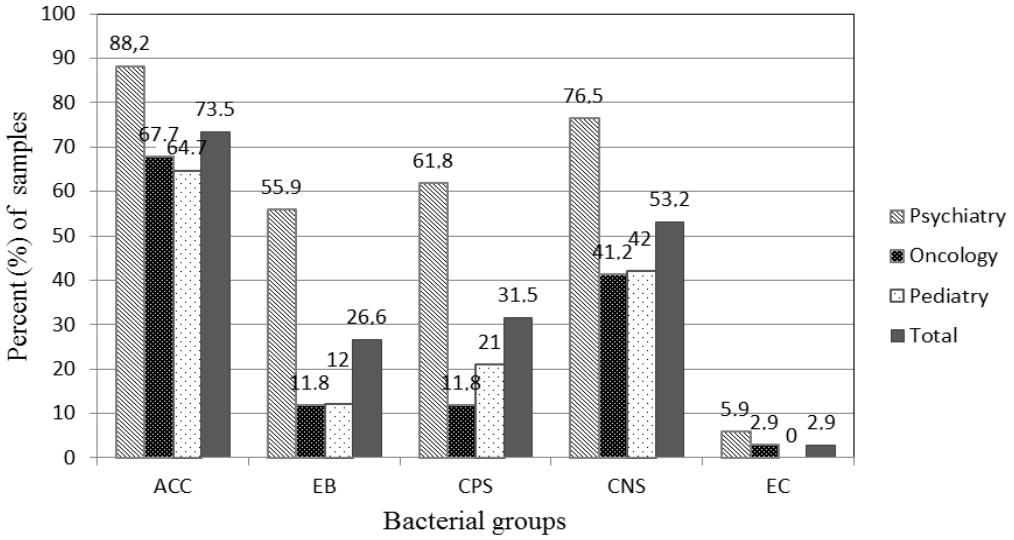

Figure 1 *Percent (%) of samples in which individual bacterial groups were detected (n=102 samples). ACC: Aerobic colony count; EB:* Enterobacteriaceae*; CPS: Coagulase-positive staphylococci; CNS: Coagulase-negative staphylococci; EC:* Enterococcus

were found on surfaces in the paediatric ward (Figure 1, Figure 2).

After biochemical identification, the most frequent isolates from the family *Enterobacteriaceae* were *Serratia marcescens* (53.8 % of isolates), *Enterobacter aerogenes* (23.1 %), *Yersinia enterocolitica* (15.4 %), and *E. coli* (7.7 %). We also detected three representatives from the genus *Pseudomonas.*

Only 19 (41.3 %) out of 46 presumptive CPS strains were confirmed as *S. aureus* with biochemical tests, while others belonged to CNS *S. caprae*, *S. warneri*, *S. hominis*, *S. epidermidis*, *S. capitis*, and *S. lugdunensis* in 11 (23.9 %), 10 (21.7 %), 2 (4.3 %), 2 (4.3 %), 1 (2.2 %), and 1 (2.2 %) cases, respectively. Other CNS strains, which formed typical colonies on Mannitol Salt Phenol Agar, were not further identified.

Susceptibility to antimicrobials

The strains of *Enterobacteriaceae* were mostly resistant to nalidixic acid and trimethoprime, and were sensitive to meropenem, cefotaxime, and cephalexin (Table 3).

According to the combined disc diffusion method, there were differences between the diameters of the inhibition zones around one or more paired cephalosporin discs, exceeding 5 mm in one out of 13 strains. With this strain, which belonged to the genus *Serratia,* the PCR analyses confirmed the gene sequences from the group CTX-9 for ESBL. The production of presumptive MBLs was detected after the addition of EDTA to the discs in two (15.4 %) of the isolates; one of them was the strain, which was also positive for ESBL (Table 3). However, the genes of the tested groups of MBLs were not confirmed in any of these isolates.

About 95.7 % out of 46 staphylococcal isolates were resistant to penicillin, 36 (78.3 %) of them showed a sharp edge zone around the penicillin discs. They were highly susceptible to ciprofloxacin, erythromycin, and clindamycin, while only half of the tested strains showed susceptibility to kanamycin with a high percentage of intermediate isolates (Table 3). Only one *S. aureus* and 2 CNS strains showed resistance to oxacillin with MIC $\geq$4 μg mL^{-1} and $\geq$0.5 μg mL^{-1}, respectively (12). Two strains were obtained from the surfaces in the psychiatric ward and one of them from the oncology ward. Five (26.3 %) out of 19 *S. aureus* strains yielded resistance to amikacin, six strains (31.6 %) to erythromycin, four (21.0 %) to ciprofloxacin, and six strains (31.6 %) to clindamycin.

VRSA was not established as the MIC values were $\leq$8 μg mL^{-1} of vancomycin at all *S. aureus* strains (12).

With the cefinase test, we detected inducible β-lactamases in 10 (76.9 %) and 30 (65.2 %) of *Enterobacteriaceae* and *Staphylococcus* isolates, respectively.

DISCUSSION

On the tested surfaces of patient rooms, other clinical facilities, and the equipment of three medical wards, mostly aerobic mesophylic bacteria (ACC) were present, followed by CNS in about 53 % of collected swabs. CPS and CNS represent part of normal human microflora on the skin, and they can be easily transferred by contact with surfaces (18). We have clearly demonstrated that the degree of environmental contamination with Gram-positive organisms was much more extensive in this environment than for Gram-negative microorganisms. It was confirmed that Gram-positive bacteria persist longer on surfaces than Gram-negative bacteria (19). Different materials also affected the adhesion of microorganisms. CNS persisted for 8-21 days on cotton, while Gram-negative *P. aeruginosa* lived for only 2-24 hours on the same surface. Neely and

Maley (20) also reported that staphylococci and enterococci sometimes survive up to 90 days on common hospital materials, such as cotton, polyester, and propylene plastic. The common nosocomial pathogens survive longer on polyethylene and polyurethane surfaces than on cotton or blend surfaces (19, 20). Both groups often cause HAIs with a higher degree of antibiotic resistance (21).

Enterobacteriaceae and enterococci are indicator microorganisms for inadequate hygienic practice. The first group does not survive long on surfaces, while enterococci do (21). *Enterobacteriaceae,* which were present in 26.6 % and *Enterococci*, detected in only 3 % of our samples, were both present mostly on surfaces in the psychiatric ward (Figure 1, Table 2).

Statistically significant differences in the mean values of the bacterial count were calculated between the samples from the psychiatric ward and the other two wards, while the differences between the mean values of the number of microorganisms from the paediatric and oncology wards were not significant.

Significant differences in the bacterial count in the samples taken in February and in September were not observed. The lowest number of positive samples, as well as the lowest number of microorganisms on surfaces were found in the paediatric ward followed by the oncology ward (Figure 1, Figure 2). The number of ACC exceeded the recommended values ≤ 250 CFU 100 cm^{-2} (17) most often in the psychiatric ward (38.2 %), while only 6 % of the samples were inadequate in the paediatric ward. The number of ACC did not exceed the limits in any of the tested samples from the oncology ward.

Highly immunosuppressed patients are hospitalised in both, paediatric and oncology wards. Children with cancer are also hospitalised in the paediatric ward. Overall, patients spend longer periods in the oncology ward than in the paediatric ward, but some of them return for several shorter therapies. Psychiatric hospitalisations are usually long and re-occurring within the same institution. The highest contamination in the psychiatric ward by comparison with other two wards is probably due to the lower vigilance of staff at cleaning and disinfecting. They assume that patients are mostly not exposed to invasive surgery interventions that could lead to infections. Patients treated in this ward are in most cases not immunocompromised or do not suffer from any severe physical disease. They are also generally more mobile than those in the other two wards making the spread of microorganisms more intensive in the environment and their unwanted colonisation more frequent. Patients have an altered mental state and are often less capable of taking care of themselves or have difficulties in understanding the instructions for the proper maintenance of personal hygiene, with which they could prevent the transfer of microorganisms in their environment.

The guidelines of the National Committee for Hospital Infections (NAKOBO) (22) describe equal standard interventions in all medical wards emphasising hand disinfection, strict contact isolation, and sampling procedures for MRSA and ESBL screening tests. Samples

Table 2 *The percent (%) of the individual sampling points in all three clinical wards in which different groups of microorganisms (n=102) were detected*

Sampling points	% of samples with individual goups of microorganisms				
	ACC	EB	EC	KPS	CNS
Telephone handsets	100	33	0	33	33
Cuff for measuring blood pressure acc. RR	67	33	0	33	33
Cart for ECG apparatus	33	0	0	17	0
Wheelchair handle	100	50	0	83	33
Medicines cart	67	17	0	50	0
Serving tray	83	33	17	50	17
Teapot handle	83	33	0	67	50
Wardrobe for clean linen (inside)	50	33	0	33	50
Tray trolley for clean linen	50	17	0	33	17
Basket for dirty laundry	83	0	0	50	50
Bedside frames	67	33	17	67	33
Bathroom sink	83	33	0	50	33
Water tap (ESBL room)	83	17	17	67	33
Night table	100	33	0	100	50
Apparatus for adjusting bed backrest	67	17	0	67	50
Door handles (ESBL room)	67	0	0	33	0
Bed lining (ESBL room)	83	17	0	67	50

ACC: Aerobic colony count; EB: Enterobacteriaceae*; CPS: Coagulase-positive staphylococci; CNS: Coagulase-negative staphylococci; EC:* Enterococcus

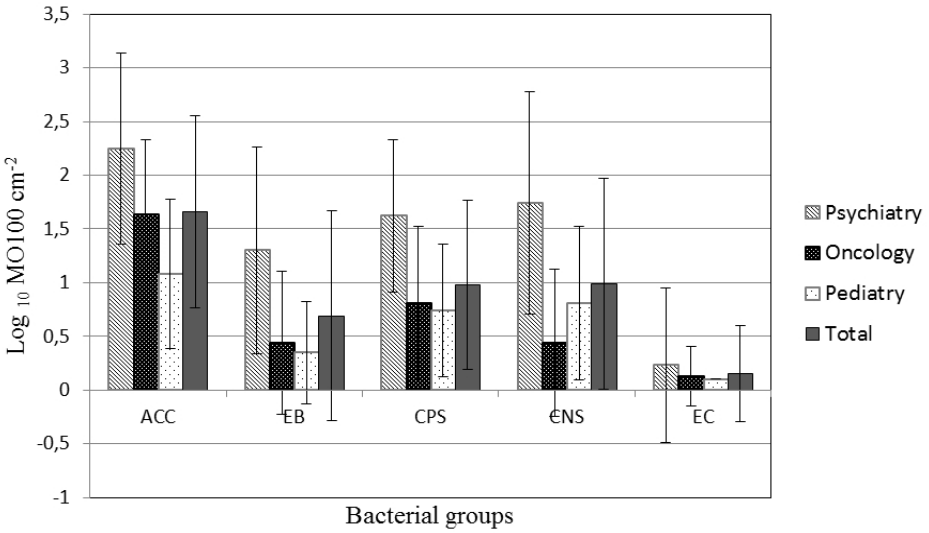

Figure 2 *The number of different groups of microorganisms on surfaces in individual medical wards. ACC: Aerobic colony count; EB:* Enterobacteriaceae*; CPS: Coagulase-positive staphylococci; CNS: Coagulase-negative staphylococci; EC:* Enterococcus*; MO: Microorganisms*

for screening are taken within 48 hours of admission to the hospital from every patient. Furthermore, controlling MRSA and ESBL infection in a psychiatric ward is specific. The contact isolation – although sometimes needed because of bacteria with multiple resistance, may cause deterioration of a patient's mental disorder. Therefore Vuga et. al. (2013) (23) recommended that specific guidelines for taking screening samples in psychiatric ward settings should be prepared.

In the psychiatric ward, greater attention should also be paid to cleaning and the appropriate choice of disinfectants. They should verify the presence of microorganisms on the hands of patients and staff. Employees should be additionally instructed about the prevention against the spreading of nosocomial infections. The routine application of rapid methods for hygiene control of surfaces is highly recommended.

In all medical wards, routine room cleaning is carried out regularly each morning and, if necessary, additionally in late afternoon according to the NAKOBO guidelines (22). In our study, the samples were not collected immediately after morning cleaning, but rather in the early afternoon, because we wished to verify not only the adequacy of cleaning but also the bacterial contamination occurring during the day. Nurses, employed in each ward, are responsible for cleaning and disinfection of the surfaces in direct contact with patients, while other equipment and sanitary facilities are cleaned by housekeepers or cleaning service staff. The type of disinfectant is prescribed specifically for individual wards (22). Additional disinfection with aerosols of orthophosphoric acid, hydrogen peroxide, and silver cations takes place in patient rooms in the paediatric and oncology wards (24), which results in lower contamination of surfaces, but according to Steinberg et al. (5) this does not always eliminate the risk of acquisition of pathogens from the environment.

Telephone headsets, night tables, wheelchair handles, serving trays, bedside frames, and water taps were the most contaminated surfaces tested in the study (Table 2). Al-Hamad and Maxwell (2008) (25) also reported that bed frames, telephones, and computer keyboards were the surfaces that yielded a high total viable count. The surfaces inside the patients' rooms (patients area) were (except the night tables) less contaminated than those in the common area of the medical ward, which was the case in our study as well.

ACC was determined in all six samplings on telephone handsets, wheelchair handles, and night table surfaces; CPS were also common on the last two mentioned objects. Brady et al. (26) studied the bacterial contamination of 70 bed-control handsets. He determined that 67 (95.7 %) of them demonstrated bacterial growth with an average log 1.5 CFU. The handsets were contaminated in our study in 67 % of cases with average log 1.6 CFU. *Staphylococcus aureus* (94.3 %), MRSA (in 12.9 %), *Enterococcus* spp. (in 41.3 %), *Bacillus* (in 5.7 %), and coliforms (in 2.9 %) of samples were mostly recognised on tested surfaces. He concluded that patients and medical and nursing staff commonly touch hospital bed-control handsets and they are relatively permanently attached to the bed frames of all inpatient beds. In our case, this is true also for surface bedside tables (Table 2). These characteristics suggest their use as a potential marker of general healthcare environmental contamination (26).

In our study, the presence of enterococci on serving trays indicated the improper washing and cleaning of the trays or inadequate hygiene by the staff delivering the meals. It is of great concern that they were also found on the water taps in the isolation rooms with ESBL positive patients

(Table 2). Neely and Maley (20) and John (27) reported that some microbes, for example, *P. aeruginosa* survived for months or longer in a wet environment, but only for a few hours to a few days on dry surfaces. We also identified three representatives from the genus *Pseudomonas,* isolated from the toilet sink in the psychiatric ward.

In our experiment, wet surfaces (water taps) were more contaminated, including with *P. aeruginosa*. Similar results were obtained by De Abreu et al. (28), where *P. aeruginosa* was repeatedly isolated from sinks and taps in different medical wards in a major hospital in Portugal.

We share the view of Pereira da Fonseca et al. (2016) (29) that strict standards of infection control in hospitals and increased public education about hand hygiene, routine schedules of cleaning, sanitising, and disinfecting are recommended to decrease the risk of transmission in hospitals among patients.

The results of Lemmen et al. (21) indicate that the inanimate environment of patients colonised or infected with multi-resistant bacteria becomes frequently contaminated, and therefore surfaces and objects may very likely serve as a secondary reservoir for cross-transmission. One of the major reasons that these organisms survive in the hospital environment is their intrinsic resistance to several commonly used antibiotics and, perhaps more importantly, their ability to acquire resistance to all currently available antibiotics, either by mutation or by receipt of foreign genetic material through the transfer of plasmids and transposons (30). In the second Slovenian national HAIs prevalence survey, conducted within the European point prevalence survey of HAIs and antimicrobial use in acute-care hospitals, 5628 patients were included. About 3.8 % of patients had at least one HAI and additional 2.6 % were still being treated for HAIs on the day of the survey; the prevalence of HAIs was 6.4 %. The prevalence of urinary tract infections was the highest (1.4 %), followed by pneumoniae (1.3 %), and surgical site infections (1.2 %) (31).

The strains from the fam. *Enterobacteriaceae,* isolated from examined surfaces in three medical wards, belonged mostly to *Serratia marcescens* (53.8 % of isolates), *Enterobacter aerogenes* (23.1 %), *Yersinia enterocolitica* (15.4 %), and *E. coli* (7.7 %). *S. marcescens* represents an important cause of HAIs, especially in neonatal intensive

Table 3 *Susceptibility of the tested isolates from the* Enterobacteriaceae *family (n=13) and genus* Staphylococcus *(n=46) using the disc diffusion method and combination disc diffusion test for confirming ESBL production*

Antimicrobial agent	Disc diffusion breakpoints[b]			% of strains			Combined disc diffusion method inh. cone (mm)		
	S	I	R	S	I	R	Range	Mean	% ≥5[c]
Enterobacteriaceae									
Cephalexin[d]	≥14		<14	53.8	0	46.2	6-30	16.1	
Ceftazidime	≥21	18-20	≤17	38.5	15.4	46.2	6-34	15.7	
Ceftazidime/CA[a]				38.5	15.4	46.2	6-35	16.1	0
Cefotaxime	≥26	23-25	≤22	23.1	23.1	53.8	6-30	17.2	
Cefotaxime/CA[a]				38.5	7.7	53.8	6-32	17.5	0
Cefpodoxime	≥21	18-20	≤17	23.1	7.7	69.2	6-25	12.9	
Cefpodoxime/CA[a]				23.1	7.7	69.2	6-28	13.4	7.7
Ciprofloxacin	≥21	16-20	≤15	38.5	30.8	30.8	6-29	17.2	
Meropenem	≥23	20-22	≤19	53.8	0	46.2	8-32	19.1	
Meropenem/EDTA				53.8	0	46.2	8-34	19.4	15.4
Imipenem	≥23	20-22	≤19	46.2	7.7	46.2	8-33	19.3	
Imipenem/EDTA				53.8	0	46.2	8-33	19.3	7.7
Nalidixic acid	≥19	14-18	≤13	30.8	0	69.2	28-40	34.8	
Tobramycin	≥17		≤14	69.2	0	30.8	31-41	37.9	
Trimethoprim	≥18		≤15	15.4	30.8	53.8	6-25	10.3	
Staphylococcus									
Benzyl penicilin	≥29	-	≤28	4.3	0	95.7	6-11	6.7	
Ciprofloxacin	≥21	16-20	≤15	91.3	2.2	6.5	6-40	27.2	
Clindamycin	≥21	15-20	≤14	84.8	6.5	8.7	6-42	27.2	
Erythromycin	≥23	14-22	≤13	80.4	6.5	13.0	6-40	26.5	
Kanamycin[d]	≥18	14-17	≤13	50.0	45.7	4.3	6-32	19.0	

[a]CA, clavulanic acid; [b](12); S, susceptible; I, intermediate; R, resistant; [c]percent (%) of strains, where there was a difference between the zone diameters of either of the antibiotic discs and their respective antibiotic/CA or /EDTA discs ≥5 mm; [d](16)

care units (32). Ivanova et al. (33) reported that *S. marcescens* was also found in the environment in their hospital, particularly on nurses' hands, suggesting transmission by staff handling.

In our study, 40 % of isolates from the family *Enterobacteriaceae* showed multiple resistance to three or more different groups of tested antibiotics. Hauser (34) found out that the strains of *Enterobacter*, *Serratia*, *Citrobacter*, *Providencia*, and *Morganella* spp. were mostly sensitive to quinolones (e.g. ciprofloxacin) and sulpha drugs (e.g. sulfamethoxazole in combination with trimethoprim), the fourth generation of cephalosporins (cefepine) and amino-glicosydes tobramycin, gentamycin, and amikacin. Carbapanems are often effective as well, while the third-generation cephalosporins (e.g. cefotaxime) should not be used to treat infections caused by these bacteria (34, 35). The isolates from the surfaces of our medical wards also showed higher sensitivity to carbapenems (about 54 %) and tobramycin (69 %) but not to trimethoprim (15 %). A higher proportion of intermediate susceptibility to trimethoprim and to ciprofloxacin (30.8 %) was also detected. The resistance of *S. marcescens* strains to tobramycin (11 %) was similar to the data (7.1 %) published by Mahlen (36). Inducible β-lactamases were found, using a cefinase test, in 73 % of all tested strains, which is in correlation with their apparent resistance to β-lactam antibiotics (Table 3). ESBL production was detected with a combined disc test in 26.6 % of strains, but the ESBL type CTX-9 was confirmed with chosen primers in only one *S. marcescens* isolate (6.7 %). Some other studies show that *S. marcescens* strains most commonly carry CTX-M-type ESBLs, particularly bla_{CTX-1}, bla_{CTX-3} bla_{CTX-9}, bla_{CTX-15} (33, 37, 38). The members of *Enterobacteriaceae,* including *Serratia* spp. and *Enterobacter* spp., to which most of our isolates belonged, carry a chromosomal ampC gene. AmpC β-lactamases inactivate a wide variety of β-lactam antibiotics and are not inhibited by clavulanic acid (36, 39). The molecular characterisation of our isolates for these sequences has not yet been performed. All *Serratia* species are also intrinsically sensitive to carbapenems, although some *S. marcescens* strains that harbour chromosomal carbapenemases have been identified (36). Plasmid-borne class B MBLs, including IMP-type variants and VIM-2, have been identified in *S. marcescens* (40). Bla_{VIM-1} gene was also detected in the representatives of the genus *Enterobacter* (41). With the combined disc method, we found only one presumptive strain producer of carbapenemases, but it was not confirmed by PCR with the chosen primer.

Invasive staphylococcal infections constitute a public health problem and a therapeutic challenge (42). In addition to *Staphylococcus aureus*, CNS are becoming significant as the causative agents of hospital-acquired infections, which are often associated with multiple antimicrobial resistance mechanisms including, in particular, methicillin resistance (43, 44). CNS in Europe were resistant to penicillin in 91 to 96 % clinical isolates, to erythromycin in 67 to 72 %, to clindamycin in 33 to 67 %, to ciprofloxacin in 79 %, and to vancomycin in 0 % (42). Our results show similar resistance of staphylococci, including *S. aureus,* to penicillin and higher susceptibility to almost all other tested antibiotics. These data are comparable with reports of the skin isolates' susceptibility from the Central Slovenia region published by Švent-Kučina et al. (45) and the isolates from the nasal mucosa of medical students in Japan (46). Švent-Kučina et al. (45) also reported about 2.8 % of MRSA strains, which is slightly lower than the results of our study (5.2 %) and the results obtained by other authors (6.9 %) (25) (Table 3).

The penicillin disc zone edge and cefinase tests were positive for β-lactamase production at 78.3 % and 65.2 % of isolates, respectively. Regardless of the 95.7 % resistance to penicillin in the tested strains, we can assume that the methods are not sensitive enough, as was also noted in previous studies (47, 48).

More samples should be collected during different seasons in future experiments for a better understanding of bacterial transmission and survival on the surfaces of medical wards. For the determination of the number and presence of individual bacterial species we used conventional culture techniques including biochemical tests, which are the limitations of our study. Therefore, we did not provide such accurate results as those enabled by the use of different PCR techniques, like illumina massively parallel sequencing approach of the 16S rRNA genes (29).

CONCLUSIONS

The aerobic colony count and coagulase-negative staphylococci were in our study most often found on the surfaces of wheelchair handles, telephone handsets, and night tables in medical wards.

The percentage of contaminated sampled surfaces was the highest in the psychiatric ward. We recommend more attention to washing hands, cleaning, and disinfecting of equipment surfaces and instruments, proper laundering, food preparation and delivery, and education of staff as well as patients and visitors.

It was a matter of concern that 40 % of the isolates from the fam. *Enterobacteriaceae* showed multiple resistance to the tested antibiotics, while MRSA was rare.

The hospital environment is contaminated by a variety of pathogenic and nonpathogenic multiple resistant bacteria that can persist on surfaces for prolonged periods. The hands and gloves of healthcare workers readily acquire pathogens after contact with contaminated hospital surfaces and they can subsequently transfer these organisms to patients and inanimate surfaces. For these reasons, hospitals must prepare a strategy for cleaning and disinfection in the wards, as well as the guidelines for reducing the risk of transmission of pathogens via contaminated hospital surfaces and

medical equipment for healthcare and medical personnel according to the recommendations of the World Health Organization (WHO) and Centers for Disease Control and Prevention (CDC).

The data obtained in our study can give us useful information about the particularities of transmission ways of microorganisms in different medical wards. Strict and regular monitoring using rapid tests for hygiene quality of surfaces, necessarily supplemented with classical microbiological methods should be implemented.

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Conflict of interest

The authors declare no conflicts of interest.

Abbreviations

HAIs: healthcare-acquired infections; MRSA: methicillin-resistant *Staphylococcus aureus;* VRE: vancomycin-resistant enterococci; ESBL: extended spectrum β-lactamase; ACC: aerobic colony count; EB: *Enterobacteriaceae;* CPS: coagulase-positive staphylococci; CNS: coagulase-negative staphylococci; EC: enterococci; CFU: colony forming units; MBL: metallo-beta-lactamase

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Raznolikost in odpornost bakterijske mikroflore na kontaktnih površinah v različnih kliničnih oddelkih

Ugotavljali smo število in prisotnost nekaterih bakterijskih skupin, vključno z ESBL in MRSA na površinah opreme in instrumentov v bolniških sobah in drugih delovnih prostorih v treh različnih kliničnih oddelkih. Število mikroorganizmov v brisih, odvzetih na izbranih površinah, smo določili z metodo štetja kolonij na selektivnih mikrobioloških gojiščih. Skupno število aerobnih mezofilnih mikroorganizmov smo našli v 73,5 % od 102 vzorcev, s povprečnimi in najvišjimi vrednostmi $2,6 \times 10^2$ in $4,6 \times 10^3$ CFU 100 cm^{-2}. Predstavnike družine enterobakterij, koagulaza pozitivne stafilokoke, koagulaza negativne stafilokoke in enterokoke smo ugotovili v 23,4, 31,4 53,2 in 2,9 % vzorcev. Število mikroorganizmov na površinah v psihiatričnem oddelku se je statistično značilno (P<0,001) razlikovalo od števila, dobljenega na vzorčenih površinah onkološkega in pediatričnega oddelka. Okoli 40 % od 19 izolatov iz družine *Enterobacteriaceae* je bilo večkratno odpornih proti trem ali več različnim skupinam testiranih antibiotikov, medtem ko je bil kot ESBL potrjen le en sev. Osamljeni sevi stafilokokov so bili večinoma odporni proti penicilinu. MRSA je bil potrjen v 5,2 % sevov *S. aureus*. Zaposleni morajo večjo pozornost posvetiti pravilnemu umivanju rok, čiščenju in razkuževanju površin, instrumentov in opreme v bolniških sobah in tudi v drugih prostorih kliničnih oddelkov, zlasti to velja za psihiatrični oddelek. Priporočljiva je uporaba hitrih metod za ugotavljanje uspešnosti čiščenja površin.

KLJUČNE BESEDE: *bolnišnice; površine; bakterijska kontaminacija; antibiotiki; občutljivost*