Official journal of the Spanish Society of Chemotherapy

Original

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Drug-resistant bacteria on hands of healthcare workers and in the patient area: an environmental survey in Southern Italy's hospital

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Article history
Received: 11 January 2019; Revision Requested: 18 February 2019; Revision Received: 19 March 2019; Accepted: 8 May 2019

ABSTRACT

Background. The WHO recognized antimicrobial resistance as a growing global health threat with a wide variability across Europe: in Italy these rates are higher than in other countries. The aim of our study was to detect antimicrobial resistance on the hands of healthcare workers and on surfaces around the patient, to assess the variability between levels of bacterial contamination on these surfaces and to compare the results with those achieved six years ago.

Material and methods. The study was conducted from June 2017 to May 2018 using contact slides for surfaces and active sampling for air. We used automated biochemical methods to identify microorganisms; antibiograms were performed in compliance with the EUCAST expert rules.

Results. We analyzed 3,760 samples, 16.17% were found positive and 34 % of these were antimicrobial-resistant. On analyzing the isolated Staphylococci, 39% were multidrug-resistant and 5% extensively drug-resistant. A 30% of the *Enterococcus faecalis* isolates were resistant to gentamycin and vancomycin. We found *Klebsiella pneumoniae* isolates resistant to ceftriaxone, cefoxitin, mecillinam and imipenem. A 7% and 8% of the *Acinetobacter baumannii* and *Pseudomonas aeruginosa* isolates, respectively, were resistant to gentamicin, imipenem, and ceftazidime

Conclusions. These findings are in line with the international literature, confirming that antimicrobial resistance is also steadily growing in Italy with rates varied for the different pathogens.

Key words: Antimicrobial Drug Resistance, Bacteria, Patients, Hospital surfaces, Healthcare workers

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Bacterias resistentes en las manos de trabajadores sanitarios y en el área del paciente: un estudio ambiental en un hospital del sur de Italia

ABSTRACT

Introducción. La OMS reconoce la resistencia a los antimicrobianos como una creciente amenaza para la salud mundial con una amplia variabilidad en toda Europa: en Italia estas tasas son más altas que en otros países. El objetivo de nuestro estudio fue detectar la resistencia a los antimicrobianos en las manos de trabajadores sanitarios y en las superficies alrededor del paciente así como evaluar la variabilidad entre los niveles de contaminación bacteriana en estas superficies y los resultados obtenidos hace seis años.

Material y métodos. El estudio se realizó entre junio de 2017 y mayo de 2018 utilizando dispositivos de contacto para superficies y muestreo activo de aire. Se empleó métodos bioquímicos automatizados para identificar microorganismos y la sensibilidad antimicrobiana fue realizada de acuerdo con las normas del EUCAST.

Resultados. Se analizaron 3.760 muestras, de las cuales el 16,17% fueron positivas y el 34% de ellas fueron resistentes a antibióticos. Al analizar los estafilococos, el 39% fueron multirresistentes y el 5% extremadamente resistentes. Un 30% de las cepas de *Enterococcus faecalis* fueron resistentes a gentamicina y vancomicina. Se aislaron cepas de *Klebsiella pneumoniae* resistentes a ceftrixona, cefoxitina, mecillinam e imipenem. Un 7% de las cepas de *Acinetobacter baumannii* y un 8% de las cepas de *Pseudomonas aeruginosa* fueron resistentes a gentamicina, imipenem y ceftazidima.

Conclusiones. Estos hallazgos están en línea con los estudios publicados en otros países, lo que confirma que la resistencia a los antibióticos también está creciendo constantemente en Italia con tasas variadas para los diferentes patógenos

Palabras clave: resistencia a antimicrobianos, bacterias, pacientes, superficies de hospitales, trabajadores sanitarios.

INTRODUCTION

Nosocomial infections are the leading cause of morbidity and mortality worldwide. According to the European Centre for Disease Prevention and Control (ECDC), the impact of six health care-associated infections (HCAIs) (pneumonia, urinary tract infections, surgical site infections, *Clostridium difficile* infections, neonatal sepsis and blood infection) is higher than the combined impact of influenza, HIV/AIDS infections and tuberculosis [1]. In Europe, HCAIs account for 37,000 deaths annually in 2014 [2]. Their economic impact is also significant amounting to approximately 9.8 billion US dollars/year for the five main infections [3]. Antimicrobial resistance is one of the main problems associated with HCAIs [4]. The Centers for Disease Prevention and Control (CDC) estimates that over two million people/year acquire antimicrobial-resistant infections, and 23,000 die as a result [5]. In Europe, 25,000 people/year die from drug-resistant infections [6]. Several studies have been published describing links between contaminated patient environments to an increased risk of HCAIs [7]. Although it is well-[established that pathogens can survive in healthcare environments for long periods of time, the exact survival times of different pathogens vary depending on certain conditions, for example temperature. The ECDC point prevalence survey of HCAIs and antimicrobial use in acute care hospitals (2011-2012) [8] ranked the most frequently isolated microorganisms from HCAIs as follows: *Escherichia coli* (15.9%), *Staphylococcus aureus* (12.3%), *Enterococcus* spp. (9.6%), *Pseudomonas aeruginosa* (8.9%) *Klebsiella* spp. (8.7%), coagulase-negative staphylococci (7.5%), *Candida* spp. (6.1%), *Clostridium difficile* (5.4%), *Enterobacter* spp. (4.2%), Proteus spp. (3.8%) and *Acinetobacter* spp. (3.6%). These pathogens are associated with HCAIs causing increases in mortality and morbidity [9].

In Italy, HCAI rates range from 5 to 10%, and infections caused by antimicrobial-resistant microorganisms are becoming more and more common, with a mortality rate of 20-30% [10]. Some studies have surveyed the incidence of HCAIs in southern Italy, including our hospital [11, 12]. The HCAI rate detected for our hospital was 4.3% (each HCAI identified in accordance with ECDC criteria) [12, 13]. Many microorganisms are involved in these infections, but multidrug resistance organism (MDROs) play a fundamental role, even in our hospital reality [14-16].

Many studies have reported the isolation of these microorganisms on hands of healthcare workers (HCWs); for example methicillin-resistant *Staphylococcus aureus* (MRSA), *Serratia marcescens* and other Gram-negative microorganisms [17-21]. On hospital surfaces some studies reported isolation of MRSA, *Escherichia coli* and *Klebsiella pneumoniae* with extended-spectrum beta-lactamases, and carbapenem-resistant *Acinetobacter baumannii* [22-23]. In light of these findings, careful monitoring of environmental contamination and analysis of the resistance profile of isolated germs is essential [24]. The aim of our study was to detect antimicrobial resistant bacteria on the hands of HCWs and on surfaces around the patient; to assess the variability between levels of bacterial contamination on different surfaces examined (Universitary Hospital of Messina, Gaetano Martino) and to compare the results with those achieved six years ago from a previous study.

MATERIAL AND METHODS

Samples were collected from the hands of HCWs and from surfaces considered at risk, namely ones near the patient and ones touched by HCWs (bed and headboard, sink, floor, med trays). A longitudinal study was conducted from June 2017 to May 2018. Samples were collected from the following wards: clinical (Cardiology, Internal Medicine, and Geriatrics), surgical (Thoracic Surgery, Orthopaedics and Vascular Surgery) and intensive care.

Contact slides (Liofilchem) were used to collect samples both for surfaces and hands of healthcare workers with a contact time of 10 seconds for the following types of culture medium used: PCA for bacterial charge, Vogel-Johnson Agar for *Staphylococcus* spp, Cetrimide Agar for *Pseudomonas* spp, Rose Bengal-CAF Yeast and Mold Agar, VRBG Agar for *Enterobacteriaceae* and Bile-Esculin Agar for *Enterococcus* spp.

All samples were taken directly to the laboratory and incubated at 37 °C for 48-72 hours.

Samples were classified as positive in accordance with the manufacturer's instructions for the contact slides (> 14 colonies on slide corresponding to 117 CFU/100 cm^2) [25, 26].

Test-positive samples were used to grow subcultures in selective agar culture media: Mannitol Salt Agar (Oxoid) was used for the isolation of *Staphylococcus* spp; MacConkey Agar (bioMérieux) was used for the isolation of Gram-negative bacteria; Enterococcosel Agar (bioMérieux) for *Enterococcus* spp; Cetrimide Agar (bioMérieux) for *Pseudomonas aeruginosa*.

Samples to assess microbial air contamination (expressed as CFU/m3) were collected from the center of the room using a semi-automatic sampler (SAS Super100, Sampler Air System, PBI), which aspirated a volume of 180 l/min. The SAS held one 55 mm diameter plate containing the different selective agar culture media (Mannitol Salt Agar, MacConkey Agar, Enterococcosel Agar and Cetrimide Agar).

Subsequently, automated biochemical methods (VITEK® 2, Bio-Mérieux, France) were used to identify microorganisms grown in subcultures.

Antibiograms were performed on the same isolated strains using MIC (minimum inhibitory concentration) and zone diameter breakpoints in compliance with the European Committee on Antimicrobial Susceptibility Testing (EUCAST) expert rules. Results were read after 24 hours by reference to EUCAST Clinical Breakpoint Tables.

MIC values were determined by spot inoculation of 1-2μL of the inoculums (~0.5 Mc Farland) on Mueller Hinton agar plates, containing different concentrations of the antimicrobial and incubated at 37°C for 18 hours. Antimicrobial susceptibility was tested according to the different bacteria: for

Staphylococcus spp. we used ampicillin, ceftriaxone, cefoxitin, oxacillin, vancomycin, imipenem, and penicillin, for *Enterococcus* spp. we employed ampicillin, gentamicin, and vancomycin, for *Enterobacteriaceae* we tested ceftriaxone, cefoxitin, ampicillin, mecillinam, and imipenem and finally for *Pseudomonas* spp and *Acinetobacter* spp we used imipenem, ceftazidime, and gentamicin. In our study, we used multidrug-resistant (MDR) and extensively drug-resistant (XDR) definitions of Magiorakos [27].

Statistical analysis. The sample was determined considering a percentage of MDR for *Staphylococcus* of 15% in our hospital estimating a 99% Confidence Interval (CI) and absolute precision of 5%. For others organisms we did not know the percentage of MDR in our hospital facilities and so we assumed the percentage of 50%. So, the minim sample size was 1,058 surfaces examined of which 49.15% (n= 520) hands of HCWs and 50.85% (n=538) environmental surfaces.

We evaluated whether antimicrobial resistance varied for the different surfaces examined, i.e. in proximity to the patient and those touched by healthcare staff. We therefore compared antimicrobial susceptibility of *Staphylococci*, *Enterobacteriaceae*, *Pseudomonas* and *Acinetobacter* on the hands of HCWs and on surfaces of the 'patient zone' (as defined by WHO). Therefore, 2x2 contingency tables were built and assumptions tested by the chi square method, while degrees of freedom were used to partition r x k tables. Also we evaluated statistical differences between the results recorded between 2012 and 2016 only for *Staphylococcus* spp. P-values of <0.05 were considered to indicate significance. Software R was used for statistical assessment [28].

RESULTS

We analysed 3,760 samples, of which 50.85% (n=1,912) were environmental and 49.15% (n=1,848) from hands of HCWs, and on total 16.17% (n=608) were positive. The positive samples for environmental surfaces were 26.57% (508/1,912) and for hands of HCWS were 5.41% (100/1,848). Table 1 shows microorganisms recovered from hands of HCWs and environmental surfaces. The percentage of isolated microorganisms with relative resistance profiles was reported in table 2.

Antimicrobial-resistant bacteria (at least resistant to one antimicrobial) were found in 33.55% (204/608) of the analysed environmental samples. These originated from the following surfaces: 40% from "frequent touch" surfaces (bed bar, washbasin, bedside table and food tray, light switch, door handle); 38% floor; 10% air; 9% medical devices and 3% HCWs' hands.

Of the 608 microorganisms identified, 55.3% were Gram-positive and 44.7% were Gram-negative, belonging to the following genera: *Staphylococcus*, *Enterobacteria*, *Pseudomonas*, *Acinetobacter*, *Rhizobium*, *Sphingomonas*, *Ochrobactrum*, *Streptococcus* spp., *Aerococci*, *Burkholderia*, *Roseomonas* and *Kytococcus*. We only analysed the first four genera for antimicrobial susceptibility because others only cause infections in immunocompromised patients. 32. 2% (196/608) of the analysed samples were found to be resistant to at least one agent in three or more antimicrobial categories (MDR).

Table 1 Microorganisms for hands of HCWs and environmental surfaces

	n	Total[a]	Hands of HCWs[a]	Environmental surfaces[a]
Staphylococcus spp.	316	51.97	5.92%	46.05%
S. aureus	32	5.27	0.66%	4.61%
CoNS	284	46.71	5.26%	41.45%
Other Gram-positive	16	2.63	2.63%	0.00%
Enterobacteriaceae	108	17.77	3.95%	13.82%
Pseudomonas spp.	52	8.55	1.97%	6.58%
Acinetobacter spp.	44	9.20	1.97%	5.26%
Other Gram-negative	40	6.58	0.00%	6.58%
Rhizobium spp.	32	5.26	0.00%	5.26%

[a]The percentage was calculated on the total samples (n=608).
HCWs = Healthcare workers. CoNS: coagulase-negative staphylococci

Gram-positive bacteria

***Staphylococcus* spp.** Isolated staphylococci accounted for 51.97% of the sample (316/608), 81% of these were coagulase-negative staphylococci (CoNS) belonging to the following species: *S. auricularis (3%), S. capitis (8%), S. caprae (1%), S. cohnii (4%), S. epidermidis (6%), S. haemolyticus (9%), S. hominis (19%), S. lugdunensis (1%), S. pasteuri (1%), S. saprophyticus (4%), S. simulans (6%), S. warnerii (5%), S. xylosus (14%).*

The remaining 19% were coagulase-positive, of which 10% were *S. aureus*. Analysis of isolated *Staphylococci* showed 54% to be resistant to at least 1 antimicrobial, 39% MDR and 5% XDR (75% *S. aureus* and 25% *S. capitis*) isolated on bedroom patient (25%) and on floor (75%) (table 2).

Other Gram-positives. *Kocuria rosea* was detected in 0.7% of samples, *Kytococcus sedentarius* was also isolated in 0.7% of samples and *Enterococcus faecalis* was isolated in 1.3% of samples. For the latter, we analysed antimicrobial resistance to ampicillin, gentamycin and vancomycin and found 30% of microorganisms to be antimicrobial-resistant.

Gram-negative bacteria

Enterobacteriaceae. An 17.77% (108/608) of the analysed samples were *Enterobacteriaceae* with 67% MDR, and 22% resistant to all tested antimicrobials isolated on floor (66.7%), bedroom patients (16.7%) and light switch (16.7%); 41% of *Enterobacteriaceae* were resistant to ceftriaxone, 44% to cefoxitin and ampicillin, 52% to mecillinam and 33% to imipenem (table 2).

Table 2 Percentage of resistance of the isolated microorganisms.

Isolated microorganism	Resistant to AMP	CTX	FOX	OX	MEL	VaN	IMP	PEN	GEN	CAZ
Gram-positive										
Staphylococcus spp.	37%	35%	24%	39%	0%	24%	0%			
S. aureus	50%	63%	50%	25%	0%	0%	38%			
S. epidermidis	40%	40%	0%	0%	0%	0%	20%			
Other CoNS	36%	48%	36%	28%	0%	9%	27%			
Enterococcus faecalis	30%					30%			30%	
Gram-negative										
Enterobacteriaceae		41%	44%		52%		33%			
Klebsiella spp.		33%	44%		56%		25%			
Proteus spp.		0%	100%		100%		0%			
Pseudomonas spp.							31%		21%	31%
Acinetobacter spp.							36%		20%	22%

AMP: ampicillin; CTX: ceftriaxone; FOX: cefoxitin; OX: oxacillin; MEL: mecillinam; VAN: vancomycin; IMP: imipenem; PEN: penicillin; GEN: gentamicin; CAZ: ceftazidime. CoNS: coagulase-negative staphylococci.

Klebsiella pneumoniae was isolated in 9.9% of the samples and resistance rates were as follows: 33% for ceftriaxone, 44% for cefoxitin, 56% for mecillinam and 25% for imipenem.

Proteus mirabilis was isolated in 0.7% of the samples and found to be resistant to all tested antimicrobials, with the exception of imipenem.

***Pseudomonas aeruginosa* and *Acinetobacter baumannii*.** *P. aeruginosa* was isolated in 8.55% of cases and 21% of the isolated microorganisms were resistant to gentamicin and 31% to imipenem and ceftazidime. *A. baumannii* was isolated in 9.2% of cases and 36% of the isolated microorganisms were resistant to imipenem, 20% to gentamicin and 22% to ceftazidime.

Other Gram-negative bacteria. The *Rhizobium radiobacter* species was isolated in 5.26% of cases. We found the following other Gram-negative species in 6.58% (40/608) of cases: *Citrobacter* spp. (15%), *Pantoea agglomerans* (3%), *Sphingomonas paucimobilis* (2%), *Ochrobactrum anthropi* (2%), *Enterobacter* spp. (1%), *Vibrio* spp. (1%), *Sphingobacterium thalpophilum* (0.7%), *Achromobacter denitricans* (0.7%), *Roseomonas gilardii* (0.7%) and *Aerococcus viridans* (0.7%). The antimicrobial susceptibility of these microorganisms was not tested because they rarely cause infection and affect mainly immunocompromised patients.

Statistical analysis. We evaluated whether antimicrobial resistance varied for the different surfaces examined, i.e. in proximity to the patient and those touched by healthcare staff. We therefore compared antimicrobial susceptibility of *Staphylococcus* spp., *Enterobacteriaceae*, *Pseudomonas* and *Acinetobacter* on the hands of HCWs and on surfaces of the 'patient zone' (as defined by WHO). No statistically significant differences emerged (table 3).

We evaluated whether antimicrobial resistance of the microorganisms investigated had changed compared with results obtained six years earlier. We observed increased antimicrobial resistance for *S. aureus* and for CoNS while a decrease in antimicrobial resistance was detected for *S. epidermidis* (table 4).

DISCUSSION

There are numerous studies demonstrating the presence of MDROs in the patient care environment. [29]. These studies typically focus on MRSA, vancomycin-resistant enterococci (VRE), *Clostridium difficile*, *Acinetobacter* spp. Other studies have evaluated the presence of other MDROs on environmental surfaces. Our research also confirmed the existence of a substantial percentage of Gram-positive and Gram-negative MDROs our hospital [30].

Contaminated inanimate surfaces and health care providers can be involved in the transmission of nosocomial infections and have been often described as the source for such outbreaks [31-34].

In our study, we noticed a high percentage of MDR for *S. aureus* and CoNS similar to previous studies [35]. In one study, MRSA was cultured from 43% of beds of individuals not known to be MRSA positive [29]. In a previous study, we found meticillin-resistant *Staphylococcus* strains in 14.7% (20/136) of samples taken from the hands of HCWs and in 35.7% (15/42) of those from hospital surfaces [30]. When we compared both studies, we found an increase in the antimicrobial resistance

Table 3 Surfaces analyzed and relative resistance profiles of microorganisms detected

Microorganisms	Surfaces analysed and relative profile of resistance				P value
	Hands of HCWs (n=100)		Patient zone (n=508)		
	R	S	R	S	
Staphylococcus aureus	50%	50%	25%	75%	0.375
CoNS	16.67%	83.33%	25%	75%	0.135
Enterobacteriaceae	50%	50%	16.67%	83.33%	0.16
Pseudomonas spp.	33.33%	66.67%	20%	80%	0.17
Acinetobacter spp.	33.33%	66.67%	16.67%	83.33%	0.8

CoNS: coagulase-negative staphylococci.

Table 4 Evaluation of the antimicrobial resistance of the isolated bacteria after six years

		2012	2018	P Value
S. aureus	OX	8%	25%	0.306
	FOX	0%	50%	NA
	VAN	0%	0%	NA
	MEL	8%	0%	NA
S. epidermidis	OX	3%	0%	NA
	FOX	1%	0%	NA
	VAN	4%	0%	NA
	MEL	13%	0%	NA
CoNS	OX	0%	28%	NA
	FOX	0%	36%	NA
	VAN	19%	9%	0.203
	MEL	29%	28%	0.878

CoNS: coagulase-negative staphylococci, NA = Not applicable
OX = oxacillin; FOX= cefoxitin; VAN = vancomycin; MEL = mecillinam

for *S. aureus* and for CoNS while we observed a decrease in the antimicrobial resistance for *S. epidermidis*. This is important because these microorganisms can spread and cause severe outbreaks especially in some high risk wards [36].

Enterococci are intrinsically resistant to a broad range of antimicrobial agents, including cephalosporins, sulphonamides and aminoglycosides at therapeutic concentrations [37]. We found *E. faecium* strains resistant to gentamycin and vancomycin confirming results in the literature. In fact, many studies have reported varying rates of VRE on hospital surfaces (13-16%) [38]. This contamination of rooms is due to not only to microorganisms spread by previous occupiers, but could also be due to transmission by HCWs, guests, objects as well as air flow and this explains the different contamination levels we detected [29, 39]. It is noteworthy that the environmental contamination rate with MRSA or VRE correlates with the number of culture-positive body sites for patients with clinical infections [40]. Thus, the evaluation of microbial contamination on surfaces in the patient's room and the evaluation of clinical antimicrobial resistance could be important in dealing with nosocomial infections.

Among the Gram-negative bacteria, the spread of carbapenem-resistant *Enterobacteriaceae* (CRE) strains in patients is frequently associated with multiple resistances to different classes of antimicrobials (pan-resistant strains) due to their high virulence and spread capacity among different patients as well as their ability to transmit by plasmids. Some studies have described that nosocomial surfaces play only a minor role in the transmission of CRE as it was seldom isolated from environmental surfaces. By contrast, our research indicated that 33% of the isolated *Klebsiella* were resistant to imipenem (and therefore a carbapenem-resistant *Klebsiella pneumoniae*). This is one of the principal CREs involved in HCAIs (the ECDC data for Europe show an increase in the spread of CRE, which is endemic in Greek, Italy, Turkey and Malta), suggesting the role of the vary surfaces to allow transient contamination of the hands of HCWs [31 ,41]. Another important issue reported in the literature is that patient zone and their furnishing of patient colonized with CRE is often contaminated by these organisms, with a reduction in contamination rates as you move away [41].

Clinically, *A. baumannii* and *P. aeruginosa*, together with MRSA, are the most common causes of HCAIs and their presence is correlated with environmental surface contamination [42]. While *P. aeruginosa* is intrinsically resistant to the majority of antimicrobial agents, some fluoroquinolones, aminoglycosides, some beta-lactams and polymyxins remain active in patients. In our study, resistance to imipenem was 36% for strains isolated from the patient environment. This microorganism can survive on several surfaces and it can therefore spread easily through a ward from one patient to another. As mentioned above, antimicrobial resistance can also support the persistence of such microorganisms, thus becoming the source of dangerous nosocomial outbreaks [43]. Similarly, carbapenem-resistant *Acinetobacter* spp. is common in Europe and in most cases is combined with resistance to fluoroquinolones and aminoglycosides. In our study, 36.4% of strains were found to be resistant to imipenem. It can also cause dangerous outbreaks and the application of meticulous environmental hygiene and strict compliance with infection control practices are vital to halt transmission. Indeed, some outbreaks have required the complete closure of units [44].

We found no statistical differences between the antimicrobial resistance of microorganisms isolated from surfaces around the patients and those from the hands of HCWs, confirming literature data that the bacteria on hospital surfaces are transferred by the hands of healthcare staff. The role of handwashing is thus vital to prevent the spread of these resistant microorganisms [45].

In literature many studies described that the healthcare area may be contaminated by bacteria from different patient zones in two ways: direct shedding from patients and via HCWs' hands. High-touch surfaces in the area of patients are contaminated with a higher rate of contamination in infected patients than from colonized ones [40].

In a cohort study, the authors investigated how frequently HCWs contaminated gloves and gowns after contact with patients [46]. For example, after one of every three interactions with a patient carrying *A. baumannii* (present in 80% of rooms from colonized patients) HCWs contaminated their gloves and gowns. *In* general, independent risk factors for HCWs contamination by MDROs were positive environmental cultures, stay in room for more than 5 minutes, performing physical examination and contact with the ventilator [46].

Limitations of our study are that we did not perform a molecular analysis and we did not evaluate the actions carried out by HCWs first of sampling.

In our study, after finding the positivity of the examined surfaces we made a signal to the health management and to the interested operative unit giving prescriptions on the environmental sanitation and on the behaviour of the HCWs. In particular, we have prescribed a more thorough cleaning of the patients zone, cleaning the surfaces with GIOALCOL 70® (hydroalcoholic solution, of 70% ethyl alcohol), and sanitizing with RELY ON VIRKON® (powdered product based on potassium peroximonosulfate to be diluted according to the indications in the technical data sheet (1g/L) alternating with STER-X 2000® (sodium hypochlorite 2.5%) or ANIOSPRAY QUICK® (hydro-alcoholic solution 55%, quaternary ammonium propionate, perfume).

For the behaviour of the operators it was prescribed the respect of the correct technique of washing and disinfection of the hands with different methods and products, according to the activities that must be carried out, by performing the 5 fundamental moments for hand hygiene present on the guidelines issued by the Ministry of Health with the use of hydro-alcoholic gel for the clutch and chlorhexidine for washing.

After prescriptions, we revaluated the contaminations both of HCWs hands that surfaces and we did not found any positive sample, confirming the role of monitoring and infection control strategies. [47]

Moreover, it was observed that the awareness of being evaluated can improve the adhesion of the HCWs ("Hawthorne effect") and of the clean staff, although this effect can still be reduced in time [48].

We hope, therefore, to reassess the results obtained over time and evaluate the reduction of MDROs and HCAI in our hospital facilities to fight antimicrobial resistance.

In fact, today we are faced with increasingly resistant germs and the weapons at our disposal are increasingly limited. Consequently, if we do not take urgent steps to remedy this problem, we will find ourselves faced with "killer germs" [49, 50] The prevention of HCAIs is the most widely used measure to keep them under control and also the most cost-effective; the savings from prevention can be as high as 5.5 billion dollars [3]. Strengthening infection control strategies such as hand washing, environmental sanitizing practices, the continuous training of physicians and specialists, correct use of antimicrobials and vaccine are all measures implemented worldwide to control HCAIs [47, 51-60].

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