Risk Factors for the Environmental Spread of Different Multi-Drug-Resistant-Organisms (MDRO)

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Research

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Abstract

Background

Substantial scientific evidence has accumulated that the contamination of environmental surfaces in hospitals plays an important role in the transmission of MDRO. To date, all studies have failed to identify the risk factors associated with environmental contamination. In this work, we aimed to evaluate, compare and identify factors associated with environmental contamination around carriers of different MDRO.

Methods

We conducted a prospective cohort study from May 2018 to February 2020. We included 125 patients admitted to Avicenne Hospital and Hotel Dieu de France de Beyrouth Hospital, fecal carriers of MDRO (ESBL-PE, CPE, VRE). For each patient, we did a quantification of MDRO in stool, a qualitative evaluation of presence of MDRO in 6 different environmental sites and collected several clinical data.

Results

ESBL-PE represented 34% of the carried MDRO, CPE 45% and VRE 21%. The most frequent MDRO species was E.coli . Contamination of at least one environmental site was observed for 22 (18%) patients. Only carriage of VanA was associated with a significantly higher risk of dissemination. Having a urinary catheter, carriage of OXA48 and E.coli were protective factors against environmental contamination. We didn't find any statistically significant difference in environmental contamination between E.coli and other Enterobacteriaceae carriers, also between ESBL-PE and CPE carriers.

Conclusions

In conclusion, our results showed that hospital environmental contamination rates are substantially higher for patients with VRE, compared to the low environment dissemination rates around ESBL-PE and CPE. Further studies on a larger scale are needed to confirm the validity of our findings.

Background

Recent years have shown an increase in the prevalence of colonization with Multi-Drug Resistant Organisms (MDRO) in the general population (1) and the increase of their incidence in hospitals. (2) The risks in terms of mortality and morbidity related to the acquisition and colonization with MDRO, justify the infection control management policies, proposed by different public health organizations all over the world. (3)

MDRO contaminated hospital environments may serve as a source of spread, either directly or indirectly through healthcare professionals. (4,5) Different studies have suggested that environmental contamination was associated with a higher risk of colonization or infection. (6) For instance, a patient

admitted to a room previously occupied by a patient who has been colonized or infected with a pathogen (e.g. Methicillin-Resistant *Staphylococcus aureus* (MRSA),Vancomycin-Resistant *Enterococcus* (VRE), *Acinetobacter* spp.) has an increased likelihood of developing colonization or infection with that pathogen. (7) On another hand, various other factors were implicated in environmental contamination, including room temperature and humidity, (8) the number of colonized body sites, (9) and stool microbial load. (10,11) Also, patients with asymptomatic colonization may be less likely to contaminate their environment than patients with the higher infectious burden associated with symptomatic infections. (12)

Detection of contamination of the healthcare environment has been mainly studied for various Gram-positive organisms. (10) Thus, several studies suggested a high risk of environmental contamination, reaching up to 64% and 94% around MRSA and VRE carriers respectively. (7,13) Among Gram-negative bacilli, the prevalence of environmental contamination with Extended-Spectrum Beta-Lactamase-Producing *Enterobacteriaceae* (ESBL-PE) was reported between 4% and 19%, depending on the ESBL-PE species type. (14,15) Moreover, *Lerner and al.* identified the existence of Carbapenemase-Producing *Enterobacteriaceae* (CPE) contamination in the patients' surroundings. (10)

Recent publications have suggested that contact isolation policies and/or individual rooms for MDRO colonized or infected patients, did not show additional benefits compared to the standard infection control policies. (16,17) However, to date, all studies have failed to identify risk factors associated with environmental contamination. In fact, most previous studies were conducted during outbreaks and/or didn't take into account confounding factors related to patients' individual risk factors of environmental dissemination. (14) Also, none of them has correlated the fecal quantification of MDRO to the risk of dissemination. In this context, in order to better target patients with MDRO at risk of dissemination, we propose in this work, to assess the overall risk of environmental dissemination of different MDRO, in a non-outbreak setting, taking into consideration the individual risk factors together with the microbiological aspect of resistance. Therefore, we aimed to evaluate, compare and identify factors associated with environmental contamination around carriers of different MDRO.

Methods

A prospective cohort study was conducted from May 2018 to February 2020 in two teaching hospitals: Avicenne Hospital, a French 500 bed hospital and Hotel Dieu de France de Beyrouth, a Lebanese 450 beds hospital. A total of 125 patients were included, 110 at Avicenne Hospital and 15 at Hotel Dieu de France de Beyrouth. All patients were older than 18-year-old and carriers of MDRO (ESBL-PE, CPE, VRE); known at admission or detected within the first 48 hours. The adopted policy for preventing the spread of MDRO in those 2 hospitals, required rectal screening for third-generation cephalosporin-resistant *Enterobacteriaceae* (3GCREB) if the patient displayed at least one of the following risk factors: (1) previous 3GCREB known carriage or infection, (2) treatment in a healthcare facility abroad during the previous 12 months of hospitalization. Furthermore, the intensive care units (ICUs) in those hospitals, screened all patients for 3GCREB on admission.

Rectal samples were collected from all patients within 48 hours after admission, using Eswab® (Copan Diagnostics®) by inserting the swab 1 cm into the rectum while rotating the swab. Following sampling, the swab was placed in the transport tube, supplied by the manufacturer, containing 1 ml of sterile Amies transport medium. In parallel, for each case, environmental samplings were realized: pre-moistened culture swabs, with a sterile saline solution, were used by the same operator for sampling by rubbing over 10 × 10 (100 cm2) calibrated areas of bed sheet at the crotch level, pillow sheet, bed bars, tablet, armchair and toilet seat. A duration of minimum 8 hours, between room cleaning and environmental sampling, was respected. During study period, the regular standard cleaning protocol for contact isolation was followed in both hospitals, in which, patient rooms were cleaned daily using moistened microfiber cloths and disinfecting detergent solutions for bathrooms and patient's environment and disposable cloths for the floors. Throughout the study, hygiene and infection prevention policies did not change. All swabs were immediately transported to the laboratory for processing and then stored at + 4 °C.

The following data were then collected: age, gender, recent stay abroad, ward and duration of hospitalization, antibiotic administration (previous during the year and/or during hospitalization), Charlson's score of comorbidities, (18) Katz's score of dependence, (19) previous hospitalization during the year, undergone surgery in the past 3 months, nursing procedures (central venous catheter, peripheral venous catheter, urinary catheter), presence of diarrhea, use of proton pump inhibitors and/or treatment with antidepressant during admission, urinary and/or fecal incontinence, documented MDRO species type and mechanism of resistance.

Microbiology

- Fecal carriage screening for MDRO:

Rectal swabs were inoculated onto 3 selective plates: ChromID® ESBL (BioMérieux, Marcy-l'Étoile, France), ChromID® CARBA SMART (BioMérieux, Marcy-l'Étoile, France) or ChromID® VRE (BioMérieux, Marcy-l'Étoile, France), for the screening of ESBL-PE, CPE or VRE respectively. All agar plates were incubated for 48 hours at 37 °C. Each morphologically unique colony was identified by Matrix-Assisted Laser Desorption/Ionization - Time of Flight (MALDI-TOF; Microflex, Bruker Daltonics, Bremen, Germany) mass spectrometry. (20) Antibiotic susceptibility testing according to Comité de l'antibiogramme de la Société Française de Microbiologie - European Committee on Antimicrobial Susceptibility Testing (CA-SFM - EUCAST) guidelines (21) was performed on each type of colony, grown on selective medias, suspected of being ESBL-PE, CPE or VRE. Confirmation of resistance was accomplished using double disc synergy tests for ESBL-PE production, β-Carba test (Bio-Rad, Marnes-la-Coquette, France) (2,22) and Xpert® Carba-R (real-time PCR, GeneXpert®-Cepheid system) for CPE and Xpert® vanA/vanB (real-time PCR, GeneXpert®-Cepheid system) for VRE isolates.

- Quantification of MDRO:

Rectal MDRO concentrations were quantified using a culture-based method. Relative abundance was calculated as follow: ESBL-PE or CPE concentration reported on the totality Gram-negative bacteria, and

VRE concentration reported on the aerobic cultured flora. Following sampling, rectal Eswab® (Copan Diagnostics®) were vortexed for 1 min at maximum speed upon arrival to the lab. The Amies transport medium containing bacteria (100 µl) were used for performing viable bacterial counts by serial 10-fold dilutions in 0.9% saline. (23) Total aerobic bacteria and total Gram-negative bacteria were quantified by direct plating on Columbia agar + 5% sheep blood (BioMérieux, Marcy-l'Étoile, France) and Drigalski agar (BioMérieux, Marcy-l'Étoile, France), respectively. ESBL-PE, CPE and VRE were quantified on ChromID® ESBL (BioMérieux, Marcy-l'Étoile, France), ChromID® CARBA SMART (BioMérieux, Marcy-l'Étoile, France) and ChromID® VRE (BioMérieux, Marcy-l'Étoile, France) plates, respectively. Viable bacterial counts were determined after 24 h of growth at 37 °C. The relative fecal abundance in MDRO was calculated by determining the ratio of CFU/ml of CPE to total Gram-negative bacteria, ESBL-PE to total Gram-negative bacteria and VRE to total aerobic bacteria and expressed as a percentage.

- Environmental sampling:

The environmental samples of the patients identified as carriers, taken at 6 different sites, were seeded on Columbia agar + 5% sheep blood (BioMérieux, Marcy-l'Étoile, France) and ChromID® VRE medium (BioMérieux, Marcy-l'Étoile, France) for VRE carriers; Drigalski agar (BioMérieux, Marcy-l'Étoile, France) with ChromID® ESBL plates (BioMérieux, Marcy-l'Étoile, France) or ChromID® CARBA SMART medium (BioMérieux, Marcy-l'Étoile, France) for ESBL-PE and CPE carriers. All agar plates were incubated for 48 hours at 37 °C. Each morphologically unique colony, on the selective chromogenic medias, was identified by MALDI-TOF mass spectrometry. Antibiotic susceptibility testing and confirmation of resistance, as described above, were performed on each colony suspected of being ESBL-PE, CPE or VRE. A qualitative evaluation of the presence of MDRO is correlated to the MDRO carried by the corresponding patient.

Statistical analysis

Results were expressed as the median (range) for continuous variables and N (%) for categorical variables. Variables showing associations at a significance level of α = 0·20 in a univariable analysis were selected for inclusion in the multivariable model and a stepwise selection was done. Statistical analysis was done with R software (version 3.2.2). All tests were two-tailed and P-values less than 0.05 (calculated by χ^2 test, Student's t test, or Mann-Whitney test) were considered significant. [http://www.R-project.org]).

Results

Patients:

During the study period, 125 patients, 72 males (58%) and 53 females (42%) were admitted: Eighty-two (66%) patients in a medical ward, 15 (12%) in surgery and 28 (22%) in ICU. The mean age was 61 years-old and the average length of hospital stay, 37 days (range between 1 and 147 days). The mean Katz and Charlson's scores were 4 and 5, respectively. Fifty-nine patients over 125 (47%) were exposed to antibiotics during their hospital stay, while 82 (66%) have received antibiotics during the year. Respectively, 94 (75%), 51 (41%) and 54 (43%) had been previously hospitalized during the year,

undergone a surgery in the past 3 months and have recently travelled abroad. Forty-seven (38%) patients had a peripheral venous catheter, 38 (30%) a central venous catheter and 60 (48%) a urinary catheter. Fifty-two (42%) patients were incontinent, 20 (16%) of urine, 10 (8%) of feces and 22 (18%) of both. Sixteen (13%) patients suffered from diarrhea, 60 (48%) were treated with proton pump inhibitors and only 4 (3%) patients were taking antidepressant.

Colonization with MDRO:

MDRO species were *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter cloacae* and *Enterococcus faecium*, isolated from 61 (49%), 34 (27%), 4 (3%) and 26 (21%) patients respectively. Resistance mechanisms of detected MDRO were ESBL in 43 (34%) patients, Carbapenemase in 56 (45%) patients; OXA48 in 52 (41%), NDM in 2 (2%) and KPC in 2 (2%); and VanA in 26 (21%) patients. The average quantity of MDRO was 1.9×10^5 CFU/µL of the Amies transport medium (range between 0.2 and 10^8 CFU/ µL) leading to a relative fecal abundance of 28% on average (range between 0.003 and 100%).

Environmental contamination:

One hundred and twenty-five patients' rooms were swabbed yielding 560 environmental samples: 125 taken from the bed sheet at the crotch level, 125 from the tablet, 121 from the bed bars, 92 from the pillow sheet, 64 from the armchair and 33 from the toilet seat. Forty environmental samples (7%) were positive for the same MDRO colonizing the corresponding patient. The most frequently contaminated sites were bed sheet at the crotch level (35%), pillow sheet (18%) and toilet seat (13%). Contamination of at least one environmental site was observed for 22 (18%) patients: 5 (23%) colonized with ESBL-PE, 6 (27%) with CPE; 5 (23%) OXA48 and 1 (5%) KPC; and 11 (50%) with VRE. Considering only carriers of *Enterobacteriaceae* MDRO species, the contamination of at least one environmental site occurred for only 11 patients over 88, resulting in a rate of 12.5%. As for VRE, among 26, 11 (42%) carriers contaminated at least one surrounding environmental site (Fig. 1).

Risk factors of environmental contamination:

The statistical analysis revealed that among all MDRO's mechanisms of resistance, only VanA was associated with a significantly higher risk of dissemination ($p = 0.0004$). Having a urinary catheter was a protective factor against environmental contamination ($p = 0.03$). Also, CPE, more specifically OXA48, carriers seem to contaminate significantly less the environment ($p = 0.03$). Moreover, *E. coli*, between all other MDRO species, appeared to colonize significantly less the patients who contaminated their environment ($p = 0.0048$) (Table 1).

Table 1
Comparative analysis of factors contributing to environmental contamination around all studied MDRO.

	Contaminated Environmental Site(s) (n = 22)	**Non-Contaminated Environmental Sites (n = 103)**	**P-value**
Male gender, n (%)	10 (43.7)	62 (60.2)	0.23
Age (an), median [IQR]	63 [53–69]	62 [54–70]	0.81
Charlson's score, median [IQR]	5 [3.5–5.5]	5 [3–6.25]	0.72
ICU admission, n (%)	4 (18.2)	24 (23.3)	0.78
Ongoing antibiotic use, n (%)	12 (52.2)	47 (45.6)	0.48
Recent stay abroad, n (%)	10 (45.4)	44 (43.1)	0.81
MDRO species, *E. coli* n (%)	5 (22.7)	58 (56.3)	**0.0048**
MDRO species, *K. pneumoniae* n (%)	5 (22.7)	33 (32)	0.45
MDRO mechanism of resistance, n (%)	5 (22.7)	38 (36.9)	0.22
ESBL	6 (27.2)	54 (52.4)	**0.03**
CPE	5 (22.7)	47 (45.6)	**0.03**
OXA48	1 (4.5)	3 (2.9)	0.56
KPC	0 (0)	4 (3.8)	1
NDM	11 (50)	15 (14.5)	**0.0004**
VanA			
Relative fecal abundance (%), median [IQR]	10.8 [0.4–52]	6 [0.2–43]	0.54
Absolut MDRO abundance, median [IQR]	2.8×10^5 [6.6×10^3– 1.8×10^7]	10^5 [1.6×10^3 – 1×10^7]	0.27
Katz's score, median [IQR]	3.5 [2.1–6]	5 [1.5–6]	0.33

	Contaminated Environmental Site(s) (n = 22)	Non-Contaminated Environmental Sites (n = 103)	P-value
Risk factors of dissemination, n (%)	5 (22.7)	11 (10.7)	0.17
Diarrhea	10 (45.4)	42 (40.8)	0.81
Incontinence	6 (27.3)	54 (52.4)	**0.03**
Urinary Catheter	7 (31.8)	31 (30.1)	1
Central Venous Catheter	6 (27.3)	41 (39.8)	0.33
Peripheral Venous Catheter	11 (50)	49 (47.6)	1
Proton pump inhibitors	2 (9.1)	2 (1.9)	0.14
Antidepressant therapy			
Previous hospitalization < 12 months, n (%)	16 (72.7)	78 (75.7)	1
Previous antibiotics use < 12 months, n (%)	15 (68.2)	67 (65)	1

When considering only *Enterobacteriaceae* species, no statistically significant difference was found in studied factors between the group of patients having contaminated their environment and those who have not (Table 2).

Table 2
Comparative analysis of factors contributing to environmental contamination around *Enterobacteriaceae* MDRO species.

	Contaminated Environmental Site(s) (n = 11)	**Non-Contaminated Environmental Sites (n = 88)**	**P-value**
Male gender, n (%)	5 (45.5)	57 (64.8)	0.32
Age (an), median [IQR]	61.5 [54.7–69.5]	64 [55–70]	0.71
Charlson's score, median [IQR]	5 [4–6]	5 [3–7]	0.83
ICU admission, n (%)	4 (36.3)	23 (26.1)	0.48
Ongoing antibiotic use, n (%)	6 (54.5)	37 (42)	0.52
Recent stay abroad, n (%)	4 (36.4)	35 (39.8)	1
MDRO species, *E. coli* n (%)	5 (45.5)	58 (65.9)	0.2
MDRO species, *K. pneumoniae* n (%)	5 (45.5)	33 (37.5)	0.74
MDRO mechanism of resistance, n (%)	5 (45.5)	38 (43.2)	1
ESBL	6 (54.5)	50 (56.8)	1
CPE	5 (45.5)	47 (53.4)	0.51
OXA48	1 (9.1)	1 (1.1)	0.21
KPC	0	2 (2.3)	1
NDM			
Relative fecal abundance (%), median [IQR]	0.6 [0.06–23.3]	7.1 [0.2–50.7]	0.32
Absolut MDRO abundance, median [IQR]	10^5 [6.8×10^3 – 5.7×10^7]	8.7×10^5 [2.6×10^3 – 1.3×10^7]	0.22
Katz's score, median [IQR]	3.5 [1.75–3.5]	4.25 [1.5–6]	0.88

	Contaminated Environmental Site(s) (n = 11)	Non-Contaminated Environmental Sites (n = 88)	P-value
Risk factors of dissemination, n (%)	2 (18.2)	4 (4.5)	0.13
Diarrhea	3 (27.3)	32 (36.4)	0.74
Incontinence	4 (36.4)	49 (55.7)	0.33
Urinary Catheter	3 (27.3)	25 (28.4)	1
Central Venous Catheter	4 (36.4)	39 (44.3)	0.75
Peripheral Venous Catheter	6 (54.5)	46 (52.3)	1
Proton pump inhibitors	0	2 (2.3)	1
Antidepressant therapy			
Undergone surgery < 3 months	5 (45.5)	37 (42)	1
Previous hospitalization < 12 months, n (%)	8 (72.7)	67 (76.1)	0.72
Previous antibiotics use < 12 months, n (%)	10 (90.9)	58 (65.9)	0.16

Consequently, there is no statistically significant difference in environmental contamination between *E. coli* carriers and carriers of other *Enterobacteriaceae* species, mainly *K. pneumoniae.* Also, patients' characteristics between carriers of *E. coli* and other MDRO *Enterobacteriaceae* species, were comparable for the majority of the studied individual risk factors (Table 3).

Table 3
Comparison of patients' characteristics between carriers of *E. coli* and other MDRO *Enterobacteriaceae* species.

	E. coli **(n = 57)**	**Other *Enterobacteriaceae* species (n = 42)**	**P-value**
Male gender, n (%)	28 (49.1)	34 (80.9)	**0.001**
Age (an), median [IQR]	59 [54–70]	66 [57.7–70]	0.23
Charlson's score, median [IQR]	5 [2–7]	5 [4–6]	0.51
Ongoing antibiotic use, n (%)	23 (40.3)	20 (47.6)	0.24
Recent stay abroad, n (%)	25 (43.9)	14 (33.3)	0.54
Relative fecal abundance (%), median [IQR]	9.3 [0.3–52.8]	3.6 [0.08–43.9]	0.30
Katz's score, median [IQR]	5.5 [1.5–6]	4 [2–6]	0.41
Previous hospitalization < 12 months, n (%)	38 (66.7)	37 (88)	**0.01**
Previous antibiotics use < 12 months, n (%)	32 (56.1)	36 (85.7)	**0.002**
BLSE-PE	24 (42.1)	19 (45.4)	0.83

Only three factors differed significantly between the two groups: male gender (p = 0.001), previous hospitalization during the year (p = 0.01), and antibiotic use during the year (p = 0.002) were found characteristics of MDRO *Enterobacteriaceae* species carriers other than *E. coli*. Additionally, our study showed no statistically significant difference in environmental contamination between ESBL-PE and CPE. Furthermore, in an attempt to compare carriers of ESBL-PE and carriers of CPE, we found similar patients' characteristics for the majority of the studied factors. Only 3 factors were found statistically significantly different: ongoing antibiotic use (p = 0.04) seem to characterize more ESBL-PE carriers, however, duration of hospitalization (p = 0.005) and recent stay abroad (p < 0.001) were more likely to be associated with CPE carriage (Table 4).

Table 4
Comparison of patients' characteristics between carriers of ESBL-PE and CPE.

	BLSE-PE (n = 43)	CPE (n = 56)	P-value
Male gender, n (%)	31 (72.1)	31 (55.3)	0.09
Age (an), median [IQR]	63 [53.5–70]	64 [57–70]	1
Charlson's score, median [IQR]	5 [3–7]	5 [4–6]	0.83
Duration of hospitalization, median [IQR]	2 [0–8]	6 [2–20]	**0.005**
Ongoing antibiotic use, n (%)	22 (51.1)	21 (37.5)	**0.04**
Recent stay abroad, n (%)	6 (13.9)	33 (58.9)	**< 0.001**
Relative fecal abundance (%), median [IQR]	4.1 [0.21–49.0]	8.3 [0.1–49.2]	0.84
Environmental contamination, n (%)	5 (11.6)	6 (10.7)	1
Katz's score, median [IQR]	4.0 [2.25–6]	3.7 [1.5–6]	0.9
Previous hospitalization < 12 months, n (%)	29 (67.4)	46 (82.1)	0.1
Previous antibiotics use < 12 months, n (%)	29 (67.4)	39 (69.6)	1

Discussion

Only 18% of the hospitalized carriers had at least one surface of their environment contaminated, half of them being colonized with VRE. Considering only carriers of *Enterobacteriaceae* MDRO species, the environmental contamination rate was as low as 12.5%. This result is consistent with some recent studies, also performed in a non-outbreak context. (14,24,25). In contrast, 11 carriers of VRE among 26 (42%) contaminated at least one surrounding environmental site. Thus, the statistical analysis revealed that the carriage of VanA is a risk factor associated with environmental contamination. This high rate of environmental contamination around carriers of VRE is in line with many previously published studies. (7) Also, only one study found a correlation between the relative abundance of VRE in feces and the percentage of positive environmental samples found. (9) Therefore, we confirm the importance of the strict infection control policies' application around VRE carriers. However, carriers of *E. coli*, between all other MDRO species and OXA48, among all MDRO's mechanisms of resistance, seem to colonize significantly less the patients who contaminated their environment. The difference observed between VRE and *Enterobacteriaceae* MDRO species could explain the high rate of acquisition and occurrence of secondary cases around VRE carriers, widely described in literature. (9,26,27)

The most frequently contaminated environmental sites were bed sheet at the crotch level, pillow sheet and toilet seat. This finding was also previously reported. In fact, several studies found that the detection

rate of different MDRO is reduced with increased distance from the carrier, with the bed surfaces being the most contaminated sites. (10,28,29)

Identifying patient factors associated with environmental dissemination within a hospital structure would make it possible to characterize those considered as high-level disseminators, allowing to better target environmental cleaning and minimize the risks of transmission. Risk factors could theoretically be related to the bacterial species itself and its ability to produce biofilms and resist, to factors associated with the host patient, such as a high degree of dependency or fecal incontinence, and/or elevated rectal abundance of MDRO. (11) Our study was able to identify only one factor clearly correlated with the risk of environmental dissemination, which is the carriage of VanA. However, three other factors were found protective against environmental contamination: having a urinary catheter, the carriage of *E. coli* between all other MDRO species and the carriage of OXA48. Interestingly, we found a high variability in the relative fecal abundance of all MDRO, but no correlation between the degree of gastrointestinal carriage and environmental contamination.

Curiously, when considering only MDRO *Enterobacteriaceae* species, we found no correlation between environmental contamination and any clinical characteristics of the carriers, particularly the load of gastrointestinal carriage, the *Enterobacteriaceae* species type and mechanism of resistance. Thus, none of the studied factors was associated with a significantly higher risk for environmental contamination, unlike *Lerner and al*., who found that high gastrointestinal concentration of CPE and fecal incontinence are risk factors for the environmental spread. (11) This contradictory result may be explained by the possibility that the dissemination of *Enterobacteriaceae* species in the environment is rather related to the bacterial species itself than its mechanism of resistance.

On another hand, not finding a statistically significant difference in environmental dissemination between carriers of *E. coli* MDRO and carriers of other *Enterobacteriaceae* MDRO species, being mainly *K. pneumoniae* species, stands in contradiction to different published studies demonstrating that the contamination is more frequent in the environment of *Klebsiella* carriers than in the environment of *E coli* carriers. In fact, those studies suggest that *Klebsiella* spp., known to form biofilms, which may be a way of surviving during long periods in the environment, have a higher persistence capacity in the environment that could account for the higher rate of cross transmission and the high potential to cause outbreaks in healthcare settings. (14,15,30,31) Our result could be explained by the possibility that the particular cleaning practices performed at the participating hospitals were adequate and had similar impact on *K. pneumoniae* and *E. coli*. However, unlike all previously published studies, our study took into consideration the patients' characteristics and individual risk factors for environmental dissemination. In fact, patients' characteristics profiles, including Charlson's score of comorbidities and Kat's score of dependence, were similar between carriers of *E. coli* and carriers of other MDRO *Enterobacteriaceae* species, being mainly *K. pneumoniae*. Since differences in risk factors' profiles between patients with ESBL- *E. coli* and ESBL- *K. pneumoniae*, have been demonstrated by *Freeman et al*., patients' individual risk factors could be confounding factors if not taking into consideration when studying environmental dissemination. (32)

Furthermore, we also haven't found any statistically significant difference in environmental dissemination between carriers of ESBL-PE and CPE. Despite the fact that antibiotic use seemed to be more frequent in the group of ESBL-PE carriers, we would have imagined that it could lead to an increase in fecal carriage, resulting in a more important environmental dissemination, which wasn't the case. Besides, according to the 2013 French national guidelines (33) and the international guidelines, (34) a strict isolation policy is applied on CPE carriers in healthcare facilities, including cohorting patients in a dedicated ward with dedicated healthcare workers and an extensive screening policy of contact patients. In case of identifying a non cohorted index patient, recommendations are to close the ward concerned and apply a screening policy to all contact patients. This strategy seems to be costly as is associated with bed closures and reduction of medical activity, for a relatively long duration of time. In addition to that, it could expose the isolated patients to a higher risk of complications as they are possibly receiving less optimal management for their medical condition, compared to non-isolated patients with the same medical condition. More recently, studies have suggested that neither single rooms, nor additional contact precautions, are necessary to control the spread of ESBL-PE. (35) Since we haven't found any statistically significant difference in environmental contamination between carriers of CPE and ESBL-PE, and knowing that CPE involve the same bacterial species as ESBL-PE and the resistance genes are also plasmid-mediated, plus, in the absence of randomized studies demonstrating the mandatory nature of cohorting recommendations, we may wonder about the usefulness of these costly policies around CPE.

The major strengths of our work are that it was conducted in a non-outbreak context and in two different hospitals. Also, our study has proposed to assess the overall risk of environmental dissemination of MDRO, by evaluation the contamination of the environment, taking into consideration the individual risk factors together with the microbiological aspect of resistance. We acknowledge our study has some limitations. First, the small sample size, with the limited number of patients who have contaminated their surrounding environment. Second, we did not audit compliance with cleaning practices during the study. Third, although our swabbing technique was carefully standardized, and equated with a widely-used standard, it may not be the optimal means of detecting MDRO on surfaces.

Conclusion

Our results showed that hospital environmental contamination rates are substantially higher for patients with VRE compared to those with ESBL-PE and CPE. We didn't find any difference in environmental dissemination between *E. coli* and *K. pneumoniae*, nor between ESBL-PE and CPE. This observation has implications for infection control practice, as identifying patients with a higher risk of dissemination could help the infection control team to better target environmental cleaning and minimize the risks of transmission. Improved understanding of the risk factors of the environment dissemination of MDRO in the hospital setting will provide an opportunity to develop new strategies to prevent the consequences of their transmission. Further studies on a larger scale are needed to confirm the validity of our findings.

List Of Abbreviations

MDRO
Multi-Drug Resistant Organisms
MRSA
Methicillin-Resistant *Staphylococcus aureus*
VRE
Vancomycin-Resistant *Enterococcus*
ESBL-PE
Extended-Spectrum Beta-Lactamase-Producing *Enterobacteriaceae*
CPE
Carbapenemase-Producing *Enterobacteriaceae*
3GCREB
Third-generation cephalosporin-resistant *Enterobacteriaceae*
ICUs
Intensive care units
CA-SFM – EUCAST
Comité de l'antibiogramme de la Société Française de Microbiologie - European Committee on Antimicrobial Susceptibility Testing

Declarations

Ethics approval and consent to participate

As the study was part of routine practice of the infection control surveillance program for prevention of healthcare associated infections in Avicenne hospital, only verbal patient consent was required. However, in Hotel Dieu de France de Beyrouth hospital, the study was approved by the ethical committee and a written informed consent was collected from participants. All procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional committee and with the 1964 Helsinki Declaration and its later amendments.

Consent for publication

In Hotel Dieu de France de Beyrouth hospital, the study was approved by the ethical committee and a written informed consent was collected from participants.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

RS performed the majority of samplings, treated the samples in the laboratory of microbiology and collected corresponding patients' data. TF helped with the sampling and laboratory work for some of the included patients. JRZ helped with collection of patients' data. BP performed the statistical work. RS and JRZ analysed and interpreted the patient data regarding the microbiology results. RS was the major contributor in writing the manuscript. All authors read and approved the final manuscript.

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Not applicable.

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Figures

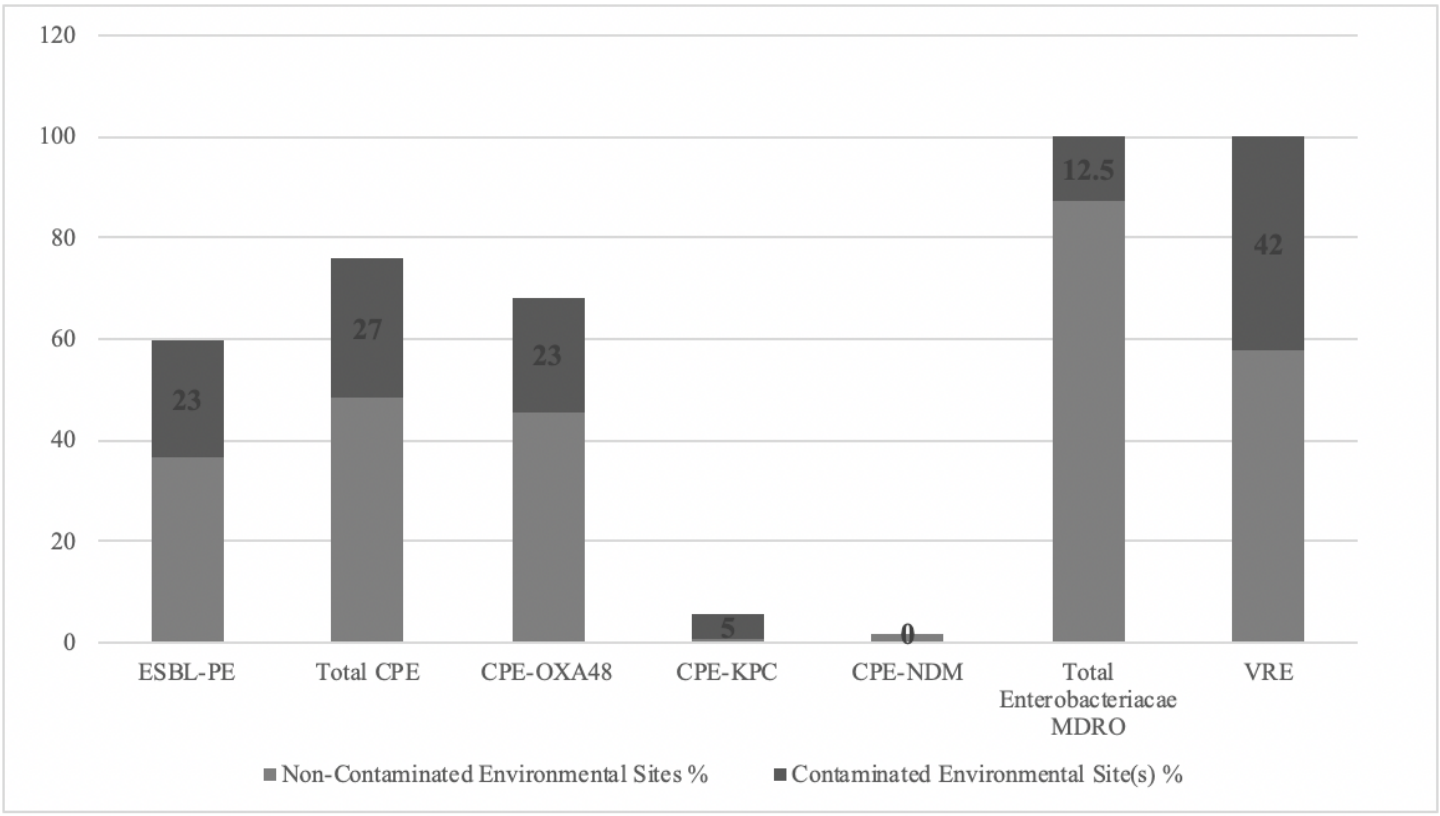

Figure 1

Repartition of patients contaminating at least one environmental site, according to the different carried MDRO. ESBL-PE: Extended-Spectrum Beta-Lactamase-Producing Enterobacteriaceae; CPE: Carbapenemase-Producing Enterobacteriaceae; MDRO: Multi-Drug Resistant Organisms; VRE: Vancomycine-Resistant Enterococcus.