Is The Disinfection Procedure of The Intensive Care Unit Able To Reduce the Bacterial Resistance and Risk of Contamination?

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Research Article

Abstract

Background: Healthcare-associated infections (HAIs) represent one of the main causes for the morbidity and mortality in the intensive care unit (ICU). In this study, we aimed to verify the presence, type, and antimicrobial susceptibility of bacteria in ICU settings, before and after disinfection procedures, and analyze the risk of contamination related to these bacteria in each area of the ICU.

Methods: The study was conducted in the ICU of a medium-sized hospital in Brazil from February 2019 to February 2020. Samples were obtained from the surfaces of beds, bathrooms, pantries, sinks, pharmacies, administrative areas, and floors, before and after the disinfection process, with 1% benzalkonium chloride and biguanide mixture (BCB). The samples were processed and inoculated in different culture media for the selective isolation of strains of clinical interest. In addition, phenotypic identification and antimicrobial susceptibility tests were performed using the VITEK® 2 system. We grouped different environmental, temporal, and microbial factors and characteristics to calculate the risk of contamination before disinfection (R_{bd}) and after disinfection (R_{ad}), as well as the total risk (R_{tt}) and real risk observed ($Fold_{risk}$) in the ICU.

Results: Gram-positive cocci and rods, gram-negative rods of the *Enterobacteriaceae* family, and non-fermenting gram-negative rods were recovered after disinfection and were found to be widely distributed. Higher bacterial diversity and frequency of resistance were observed, mainly the resistance of gram-positive bacteria to cephalosporin ($p < 0.05$) and lincosamides ($p < 0.0001$), and gram-negative bacteria to quinolones ($p < 0.0001$) and aminoglycosides ($p < 0.05$). The pantry, pharmacy, and beds showed major risks of contamination after disinfection (R_{ad}), ranging from intermediate to very high-risk levels. $Fold_{risk}$ for the pantry and beds presented a significant increase in two and three risk levels, respectively, after the disinfection process.

Conclusions: Our results demonstrate the presence of bacterial pathogens with multidrug-resistant profiles after disinfection with a higher risk of contamination, indicating that challenges still exist in the deployed disinfection protocols in the ICU settings, which are associated with the increased critical risk of HAIs after the disinfection procedure.

Background

Healthcare-associated infections (HAIs) are acquired during care or hospitalization procedures and represent one of the most critical public health problems worldwide, as they are associated with increased morbidity, mortality, antimicrobial resistance, length of hospital stay, and financial costs [1,2].

The World Health Organization (WHO) has recently listed the ESKAPE group (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa,* and *Enterobacter* species) in the list of bacteria against which new antibiotics are urgently needed [3,4].

Antimicrobial resistance is a significant threat to public health [5] and has the potential to cause 10 million deaths annually up to 2050 if nothing is done to mitigate its continued advance worldwide [6,7].

The misuse as well as excessive and uncontrolled use of antibiotics has played a vital role in selecting the antibiotic-resistant bacteria [8–10]. In intensive care units (ICUs), patients in critical and debilitating conditions are at an increased risk of being affected by HAIs [2,11]. The spread of HAIs is complex and multifactorial; therefore, strategies that aim to reduce the contamination and spread of resistant microorganisms can impact the occurrence of HAIs [12,13]. The existence of microbial resistance profiles, especially methicillin and vancomycin resistance, expression of extended-spectrum beta-lactamases and carbapenemases, and increasing resistance to macrolide agents, have become a threat in clinical practice in the ICU [14].

In this context, inanimate surfaces and devices present in hospitals serve as shelters for the most diverse microorganisms. When there is any failure in the disinfection of the environment, they can quickly spread throughout the health team and among patients, serving as a source of contamination and contributing to the cases of HAIs [15–17]. Therefore, strict cleaning protocols are used as the primary procedure to reduce contamination risks [1].

Therefore, to contribute data that can serve as guides for the development of HAI control strategies, the objective of this study was to analyze the microbial profile of the ICU in a hospital in Ilhéus (Bahia, Brazil) before and after performing the disinfection procedure on the floors and surfaces, to analyze the impact of the standard cleaning procedure established in the hospital, with particular attention to microorganisms associated with nosocomial infections and the phenotypic profiles of resistance to clinically used antimicrobials, and to analyze the risk that these bacteria represent to the patients and staff in the ICU.

Methods

Study setting and design

The study was carried out in an ICU of a medium-sized hospital in Ilhéus, Bahia, Brazil, from February 2019 to February 2020. The evaluated ICU houses critically ill patients of all medical specialties. This ICU accommodates 20 electric beds and four sections sharing the same geographical, administrative, and other facilities (patients' bathrooms, pantries, etc.). At the time of this investigation, visitors were allowed from 2 pm to 4 pm, and no limit to visitors was stipulated.

A 1% mixture of benzalkonium chloride and biguanide (BCB) was used as a disinfectant. The commercial product (BCB) is composed of 5.2% alkyl dimethyl benzyl ammonium chloride (benzalkonium chloride), 3.5% polyhexamethylene biguanide (PHMB), non-ionic surfactant, solvent, and water. The disinfection process was performed at least three times a day by diluting BCB at 1% at the time of use.

Samples were collected in the morning at the beginning of each 24 h shift, before the cleaning and disinfection of floors and surfaces and immediately after one of the sanitizing procedures. As part of the procedure, the product was left in contact with the surfaces for at least 10 min after application and to obtain the sample when there was no more humidity over the area. During sampling, all ICU employees and facilities were fully operational. Collections were performed twice per week.

Sample collection

A total of 35 paired samples (total 70) were collected from different areas and surfaces of the ICU, including occupied beds (n = 12), administrative spaces, bathrooms, pantries, sinks, and pharmacies.

Samples were collected from six bed locations (Cardiac monitor (n = 6), bed (n = 6), accessory preparation table (n = 6), bed floor (n = 6), and sink (n = 3)) and nine from common use areas. In the other areas of common use, samples of bathroom tile (n = 1), bathroom floor (n = 1), refrigerator (n = 1), microwave (n = 1), pantry floor (n = 1), pharmacy bench (n = 1), glass located above the pharmacy bench (n = 1), pharmacy floor (n = 1), and administrative area floor (n = 1) were collected (Fig. 1). All collected samples were paired before and after disinfection.

The floor samples were collected with sterile SCOTT DURAMAX sheet (Kimberly-Clark, USA), moistened with distilled water, and conditioned in sterile Zip Lock plastic bags (Talge, Brazil). In the other places (heart monitor, bed, table, sink, tile, refrigerator, microwave, bench, and glass), collections were made with a sterile swab (Absorb, China) containing Stuart medium through firm movements to ensure complete contact with the area. The collection surface covered an area of 400 cm².

All collected material was identified and kept at 4–8 °C until the beginning of processing, which took place within a maximum period of one hour.

Sample processing of samples collected in the hospital in Ilhéus-BA.

In the Laboratory of Microbiology at the State University of Santa Cruz, each sample was individually immersed in tubes containing the brain heart infusion (BHI) broth (HiMedia, India) and subjected to serial dilutions. After a 24 h incubation period at 37 °C in aerobiosis, samples were subcultured in the selective media: eosin methylene blue (EMB) agar (Merck, Germany), nutrient agar (KASVI, Brazil), and blood agar (MicroMed, Brazil). After an incubation period of 24 h at 37 °C, the isolated strains were subjected to standard tests for biochemical-physiological identification and evaluation of their morphotintorial aspect using Gram stain. Bacteria were stored at −20 °C in Luriam Bertani Miller broth (Kasvi, Brazil) with 20% glycerol.

For the samples collected using a sterile humidified Scott Duramax sheet, 1 mL of water was squeezed from the sheet and transferred to 9 mL for serial dilution. All procedures were performed under aseptic and level 2 biosafety conditions.

After the presumptive identification of bacterial strains via traditional cultivation techniques and biochemical tests, confirmation of phenotypic identification and automated antimicrobial susceptibility determination was performed using the VITEK® 2 system (BioMéreiux, Brazil) with GP ID (gram-positive cocci), ANC ID (*Corynebacterium* and anaerobic bacteria), GN ID (gram-negative rods), and AST (antimicrobial susceptibility determination) cards. The interpretation of results followed the guidelines proposed by the Clinical and Laboratory Standards Institute (CLSI) and the Brazilian Committee on Antimicrobial Susceptibility Testing (BrCAST) [18], according to the Brazilian Secretariat of Health Surveillance, resolution No. 64, on December 11, 2018 [19] .

Risk analysis

To understand the risk that the ICU microbial diversity represents to the patients and staff before and after the disinfection process, we grouped different environmental, temporal, and microbial factors and characteristics, according to the water risk analysis protocol by [20], and adapted the principle of this analysis to carry out the risk analysis of the ICU environment in this study. The following aspects were considered in the risk analysis: (i) the microbial diversity found; (ii) the number of isolates; (iii) the temporal variation in microbial diversity and quantity, namely the microbial profile before and after the ICU disinfection process; (iv) the susceptibility profile, if sensitive and intermediate (common) or resistant (resistant) isolates based on the minimum inhibitory concentration (MIC) values; (v) the location of the bacterial isolates in the ICU environment, including the locations and surfaces from which the samples were collected; and (vi) the classification of these data according to risk categories: level I = very low; level II = low; level III = intermediate; level IV = high; and level V = very high. The elements above were used to calculate the risk of contamination before and after disinfection. Then, "risk analysis," or simply "R," allowed us to predict temporal risks: before (R_{bd}) and after (R_{ad}) the disinfection process and total risk (R_{tt}), the latter being the sum of the risks before and after disinfection.

Given the differences and clinical significance of the isolates, different multiplier values were established to calculate the risk for common and resistant isolates, and for bacteria isolated before and after the disinfection process, so that the resistant isolates and isolates after disinfection have a higher calculation weight, as described in the formulae below:

Formula 1: Risk before disinfection (R_{bd})

$$R_{bd} = ((I_{c1} \times R_a) \times (I_{r1} \times R_d)) \times B_c$$

where, I_{c1} and I_{r1} represent the number of common and resistant isolates before disinfection, respectively; R_a and R_d are risk multiplier for common bacteria and resistant bacteria, whose values correspond to 0.1 and 1, respectively; B_c represents the risk multiplier value before disinfection, whose value is 1.

Formula 2: Risk after disinfection (R_{ad})

$$R_{ad} = ((I_{c2} \times R_a) \times (I_{r2} \times R_d)) \times B_d$$

where, I_{c2} and I_{r2} represent the number of common and resistant isolates after disinfection, respectively; R_a and R_d are risk multiplier for common bacteria and resistant bacteria, whose values correspond to 0.1 and 1, respectively; B_d represents the risk multiplier value after disinfection, whose value is 2.

Formula 3: Total risk (R_{tt})

$$R_{tt} = R_{bd} + R_{ad}$$

where, R_{bd} represents the risk before disinfection, and R_{ad} the risk after disinfection.

Subsequently, five risk levels were established based on the possible temporal risk values (R) before disinfection, after disinfection, and total risk in the studied ICU environment: very low risk = R < 1.0, low risk = R ≥ 1.0 ≤ 2.5, intermediate risk = R > 2.5 ≤ 5.0, high-risk = R > 5.0 ≤ 9, and very high-risk = R > 9.0. To obtain the real risk observed after ICU disinfection, we calculated the ratio between the risk after disinfection (R_{ad}) and the risk before disinfection (R_{bd}).

Formula 4: Real risk observed ($Fold_{risk}$)

$$Fold_{risk} = \frac{R_{ad}}{R_{bd}}$$

Finally, to obtain an adjusted parameter, the $Fold_{risk}$ value obtained for each ICU environment was adjusted to the previously established risk categories (very low, low, intermediate, high, and very high) to analyze whether the increase (or decrease) in the real risk observed ($Fold_{risk}$) resulted in a change between the different risk categories established. For example, if before disinfection, the pantry floor had a risk level of R_{bd} = 0.8 (very low), and after disinfection, a risk level of R_{ad} = 2.0 (low), then this environment will have a $Fold_{risk}$ = 2.5, that is, the ICU pantry floor after disinfection will have a 2.5x greater risk than before disinfection. At the same time, it will no longer belong to the category of very low risk and move to the low-risk category. Thus, when a change in the risk category was observed, as in the example mentioned above, the *risk* was considered significant. The $Fold_{risk}$ was considered significant: if the risk level decreased one level (˙); if decreased 2 levels (˙˙); if decreased 3 levels (˙˙˙); if decreased 4 levels (˙˙˙˙); if increased 1 risk level (⁺); if increased 2 levels (⁺⁺); if increased 3 levels (⁺⁺⁺); and if increased 4 levels (⁺⁺⁺⁺). When there was no change in the risk level, the *risk* was considered non-significant (#). Thus, in the example above, we would have a $Fold_{risk}$ = 2.5, with a significance level of (⁺) for the pantry floor after disinfection.

Statistical analysis

Statistical analyses were performed using GraphPad Prism Software version 7.04 (San Diego, USA). The Kolmogorov-Smirnov test was used to analyze the distribution of normality before choosing the appropriate statistical test. The frequency distribution is described as a percentage. The analysis between two groups with non-parametric distribution was performed using the Mann-Whitney test. Data are

represented as the mean ± standard error of the mean (SEM) or mean ± min to max values, and values of $p < 0.05$ were considered to be statistically significant.

Results

Disinfection process increases the microbial diversity in the ICU

Gram-positive cocci and rods, gram-negative rods of the *Enterobacteriaceae* family, and non-fermenting gram-negative rods were recovered after disinfection and were widely distributed. From the 70 samples collected, 119 isolates of bacteria were recovered, with at least one bacterium per sample. Beds (n = 44) and pantries (n = 17) were the areas with the highest numbers of bacteria (**Fig. 2**). For pantry, most of them were isolated before disinfection (n = 12 before; n = 5 after), and for bed, most of them were isolated after disinfection (n = 15 before; n = 29 after). It is noteworthy that from all the gram-positive rods, only *Corynebacterium jeikei* could be identified at the species level using the adopted methodology.

Considering all bacteria recovered before and after the sanitization process, gram-positive bacteria were the ones that prevailed (n = 65). Between them, especially before the sanitization procedure, gram-positive rods were the most frequently (54.62%) observed. Before disinfection, they were isolated from all bed and surfaces (floor, accessory T-serving table, and cardiac monitor). After the disinfection procedure, they were isolated only from the accessory T-serving table and cardiac monitor (**Fig. 2**). With the exception of the sink, *K. pneumoniae* was isolated from all areas.

After the disinfection process, all surfaces tested showed higher microbial diversity. This also included a higher frequency of phenotypic resistance among the microbes (**Fig. 2**).

Bacteria isolated after the disinfection process present a resistant profile to antimicrobial drugs

To understand the susceptibility profile, we analyzed the MIC values of different classes of antimicrobial drugs used against the bacteria isolated from nosocomial environments and analyzed the individual drugs. Regarding the antimicrobial classes in gram-positive bacteria, a resistance profile to cephalosporin ($p < 0.05$) and lincosamides ($p < 0.0001$) prevailed after disinfection (**Fig. 3A**), and a susceptibility profile against lipopeptide ($p < 0.05$) and oxazolidinones ($p < 0.01$; **Fig. 3A**) was observed. Gram-negative bacteria showed antimicrobial resistance and significant variation in MIC values after disinfection with quinolones ($p < 0.0001$) and aminoglycosides ($p < 0.05$), when compared to isolates after disinfection (**Fig. 3B**), and a susceptible profile for polymyxins ($p < 0.01$) after disinfection (**Fig. 3B**).

A more intense change in susceptibility profile based on MIC values was observed for nitrofurantoin (NIT) and trimethoprim/sulfamethoxazole (SUT) for gram-positive bacteria (**Fig. 4A**). Unlike gram-positive isolates, we observed a greater variation between MIC values of antimicrobials used in isolates before and after the disinfection process, especially for ampicillin (AMP), ampicillin/sulbactam (APS), piperacillin/tazobactam (PIT), cefuroxime (CRX), cefuroxime axetil (CRX/AXETIL), cefoxitin (CFO), ceftazidime (CAZ), ceftriaxone (CRO), and cefepime (CPM; **Fig. 4B**).

Disinfection process increases the risk of contact with pathogenic bacteria in the ICU

Risk analyses showed a higher risk of contact/exposure (and possible contamination) with bacterial isolates from the ICU settings after the disinfection process, which was associated with an increased number of resistant strains in the environment. When the risk was analyzed for each location and surface, the administrative area, bathroom, and ICU sink presented very low or low contamination risks before (R_{bd}) and after (R_{ad}) disinfection, while the pantry, pharmacy, and beds showed major risks of contamination after (R_{ad}) disinfection process, being intermediate to very high-risk levels (**Fig. 5A**). In general, 85.7% of the areas and surfaces had a smaller R_{bd} (0.2−0.5) with a very low risk level (**Fig. 5B**); however, these percentages decreased to 35.7% for very low risk level, and 21.4% and 14.3% of ICU areas present an intermediate or very high-risk level to R_{ad} (2.8−10.8; **Fig. 5C**). Evaluating the risk of contamination independent of the disinfection situation, it was shown that approximately 42.8% of the total risk (R_{tt}) to which patients and professionals are exposed in the ICU environment, represent intermediate, high, and very high-risk levels with R_{tt} values varying from 3.1−11.1 (**Fig. 5D; Table 1**).

We considered making the risk analysis more robust and for that we assessed the real risk observed (named $Fold_{risk}$), the ratio between the risk after disinfection and that before disinfection and considered the $Fold_{risk}$ significant only if a change in the risk level was noted (see **Methods**, session **Risk Analysis**). The values of the risk analysis and their significance are listed in **Table 1**. The $Fold_{risk}$ was significant for different ICU locations (**Fig. 6**). Thus, it was possible to observe that for different places evaluated, there was an increase in the real risk of contamination by bacterial isolates after the usual disinfection procedure in the ICU, especially for the pantry and beds with an increase in the two risk levels ($Fold_{risk}$ = 3.7[++]) and three risk levels ($Fold_{risk}$ varying from 5.6[+++] to 36.0[++++], with a median of 17.9[+++]) (**Table 1**; **Fig. 6**). These data suggest that the disinfection process with BCB was not efficient in decreasing the contamination risk in the ICU studied. The only place in the ICU area where the disinfection process was very efficient and did not show a change level was the sink surface ([#]).

Discussion

Environmental contamination associated with nosocomial infection risk has been increasingly observed [21,22]. It is well reported that most nosocomial pathogens may remain on inanimate surfaces for months. In this context, the emergence of resistance to disinfectants is a fact, as well as cross-resistance to antimicrobials [23−26].

Here, we report the persistence of contamination with multidrug-resistant bacteria on surfaces after disinfection. Although many aspects can be involved in the persistence of bacteria in this environment, the use of 1% BCB must be considered. Most reports available in the literature show the effect of these substances alone and in other environments, such as food industries, cosmetics, and water treatment, among others [27−30]. The results suggest that disinfection of surfaces with high bacterial

contamination may lose its effectiveness. In addition, a study performed in an ICU with the same disinfectant suggested bacterial adaptation to the environment [1].

It is essential to note the presence of multidrug-resistant profiles of some bacteria recovered from the ICU surfaces, such as ESKAPE-type bacteria, such as *Enterobacter cloacae* and *K. pneumoniae* [31]. Their presence represents a significant challenge for nosocomial infections in hospitals. While the ESKAPE phenotype, for example, can survive for long periods in the environment and characterizes a continuous source of contamination, thereby making effective therapeutic strategies difficult [24,32–35], the others show resistance related to the most common antimicrobials used for critical infection treatment, such as nitrofurantoin (NIT) and trimethoprim/sulfamethoxazole (SUT), ampicillin (AMP), and ampicillin/sulbactam (APS) [14,36–38].

New investigations must be conducted to identify the cause of the resistant bacteria recovering from this ICU investigated here; however, some facts already reported in the literature may also be involved. The prescription and excessive use of antimicrobials associated with the adaptive resistance mechanism of bacteria, several broad-spectrum agents are continually used at the same time, and the lack of antimicrobial interchange or cyclic usage to treat nosocomial infections are some of them [39,40].

We were able to show that the microbial diversity within the ICU changes and may present different profiles over time before and after disinfection. Microbiological analyses allow us to assess the microbial diversity in the ICU environment to which patients and hospital staff are potentially exposed, before and after the disinfection process; however, it is important to understand the possible impact of this diversity, which is the risk that microbial diversity found in the ICU is. Therefore, bacterial characteristics, whether the isolates are composed of pathogenic bacteria, their susceptibility profile, and identification of resistant isolates, among others, are crucial [41].

We proposed a risk analysis (R) that grouped different environmental, temporal, and microbial factors and characteristics. Based on these analyses, the data showed that the risk before the disinfection process (R_{bd}) was generally lower than the risk after disinfection (R_{ad}), and that accumulating risk or total risk (R_{tt}) varied between very low and very high in the studied ICU. The real risk observed ($Fold_{risk}$) was incredibly high to the pantry floor ($Fold_{risk}$ = 3.7) and to all bed surfaces ($Fold_{risk}$ varying from 5.6 to 36.0 with media of 17.9) of ICU after disinfection process, indicating that these areas represent a risk to the health of patients and/or staff professionals, and that disinfection processes need to be reviewed and improved to minimize the risk of surviving pathogenic bacteria persisting and may contact humans. An important limitation is that our risk analysis (R), however, did not evaluate the presence of other microorganisms that may be present in the ICU, such as fungi.

In the ICU, the risk of contamination showed that the disinfection process used did not eliminate the presence of common and multidrug-resistant bacteria in the environment. On the contrary, our evaluation showed an increase in bacterial contamination, with a high presence of multidrug-resistant bacteria after disinfection. In particular, beds, pharmacies, and pantry areas have a risk of contamination. It is

important to note that although the presence of multidrug-resistant bacteria could be detected after the disinfection process, this does not mean that they appear as a direct result of the disinfection process used, because we cannot rule out the microbial exchange that takes place between health and cleaning professionals and the ICU environment [41,42,46]. Recontamination after the cleaning protocol, frequent manipulation of the devices because the team was active during sample collection, or the resistance of microorganisms to environmental changes and stress [1,43–45] should be considered as they may increase the risk of bacterial contamination in the ICU.

In this study, there is evidence that the disinfection process used may lead to bacterial resistance selection, since a more significant number of resistant bacteria were recovered after the disinfection process. In addition, exposure of bacteria to sublethal concentrations of biocidal agents can activate adaptive stress response mechanisms, such as efflux pumps, favoring survival in hospitable environmental conditions and the emergence of bacteria that are resistant to the main antimicrobial agents used in the therapy of infectious diseases [46,47].

Conclusions

In conclusion, our data demonstrate the persistence of bacterial pathogens of clinical interest with multidrug-resistant profiles in different areas of the studied ICU, even after disinfection with BCB. In addition, the pantry floor and beds were found to be the major risk areas for ICUs. Therefore, further studies are necessary to decipher the roles of these biocides used in the hospital in the development of the wide profiles of bacteria recovered from the respective surfaces as well as the possibility of these bacteria forming biofilms. These data will help to control the nosocomial infection levels in the ICUs of hospitals.

Abbreviations

AST: Antimicrobial susceptibility determination; BCB: 1% of benzalkonium chloride and biguanide mixture; BHI: Brain heart infusion; BrCAST: Brazilian Committee on Antimicrobial Susceptibility Testing; CLSI: Clinical and Laboratory Standards Institute; EMB: Eosin Methylene Blue Agar; ESBL: Broad Spectrum Beta lactamases; ESKAPE: *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa,* and *Enterobacter* species; HAIs: Healthcare-associated infections; ICU: Intensive care unit; KPC: *Klebsiella pneumoniae* Carbapenemase; MIC: Minimum inhibitory concentration; MLSB: Macrolide agents, lincosamides and streptogramin B resistant;*Staphylococcus aureus;* PHMB: polyhexamethylene biguanide; R_{tt}: Total risk; R_{ad}: Risk after disinfection; R_{bd}: risk before disinfection; *Fold*$_{risk}$: Real risk observed; SEM: Standard error of the mean; AMC: Amoxicillin/clavulanic acid; AMI: Amikacin; AMP: Ampicillin; APS: Ampicillin/Sulbactam; CAZ: Ceftazidime; CFL: Cephalothin; CFO: Cefoxitin; CIP: Ciprofloxacin; CLI: Clindamycin; COL: Colistin; CPM: Cefepime; CPT: Ceftaroline; CRO: Ceftriaxone; CRX: Cefuroxime; CRX/AXETIL: Cefuroxime Axetil; DAP: Daptomycin; ERI: Erythromycin; ERT: Ertapenem; GEN: Gentamicin; IPM: Imipenem; LEV: Levofloxacin; LNZ: Linezolid; MPM: Meropenem; NAL: Nalidixic Acid; NIT: Nitrofurantoin; NOR: Norfloxacin; OXA:

Oxacillin; PEN: Benzylpenicillin; PIT: Piperacillin/Tazobactam; RIF: Rifampicin; SUT: Trimethoprim/Sulfamethoxazole; TEC: Teicoplanin; TIG: Tigecycline; VAN: Vancomycin; WHO: World Health Organization.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

PO and LD conceived and designed the study. PO performed the collection and microbiological analyses. PO, LD, AO, RR, RF and US analyzed the data. US performed the statistical analyses and developed the risk analysis. PO, AC, LD, AO, MS, JC, CC, GR, AP, RR, CR and US drafted the manuscript. The other co-authors (AC, AS, HP, RF, JL, RR, JC and GR) contributed to the design of the study. APM, JL HP, AS supported the ICU staff for the collection of environmental samples. All authors agree with the submission of this article. All authors read and approved the final manuscript.

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References

1. Ribeiro LF, Lopes EM, Kishi LT, Ribeiro LFC, Menegueti MG, Gaspar GG, et al. Microbial community profiling in intensive care units expose limitations in current sanitary standards. Front Public Health. 2019;7(AUG):1–14

2. Gómez-De Rueda F, Martinez-Nogueras R, Tena-Sempere ME, Elosegui-Horno I, Robles-Rodríguez L, Horno-Ureña F. Epidemiological aspects and prevalence study of nosocomial infections in a general hospital of specialties: Retrospective analysis 2012–2017. Eur J Hosp Pharm. 2019;26(6):339–42

3. Tacconelli E, Carrara E, Savoldi A, Harbarth S, Mendelson M, Monnet DL, et al. Discovery, research, and development of new antibiotics: The WHO priority list of antibiotic-resistant bacteria and tuberculosis. Lancet Infect Dis. 2018;18(3):318–27

4. World Health Organization. Antimicrobial resistance: Prioritization of Pathogens to guide Research and Development of New antibiotics [Internet]; 2017. [cited 2020 Feb 8]. Retrieved from: https://www.who.int/medicines/areas/rational_use/prioritization-ofpathogens/en/

5. Patil A, Banerji R, Kanojiya P, Koratkar S, Saroj S. Bacteriophages for ESKAPE: Role in pathogenicity and measures of control. Expert Rev Anti-Infect Ther. 2021;19(7):845–65. https://doi.org/10.1080/14787210.2021.1858800

6. Mahfouz N, Ferreira I, Beisken S, von Haeseler A, Posch AE. Large-scale assessment of antimicrobial resistance marker databases for genetic phenotype prediction: A systematic review. J Antimicrob Chemother. 2020;75(11):3099–108

7. O'Neil J. Review on antibiotic reisistance. Antimicrobial Resistance: Tackling a crisis for the health and wealth of nations. Heal Wealth Nations. 2014 (December):1–16. Retrieved from: https://amr-review.org/sites/default/files/AMR Review Paper -. Tackling a crisis for the health and wealth of nations_1.pdf

8. Finley RL, Collignon P, Larsson DGJ, Mcewen SA, Li XZ, Gaze WH, et al. The scourge of antibiotic resistance: The important role of the environment. Clin Infect Dis. 2013;57(5):704–10

9. Jensen CS, Nielsen SB, Fynbo L. Risking antimicrobial Resistance: A One Health Study of antibiotic Use and Its Societal Aspects BT - Risking antimicrobial Resistance: A collection of one-health studies of antibiotics and its social and health consequences. In: Jensen CS, Nielsen SB, Fynbo L, editors. Cham: Springer International Publishing; 2019. p. 1–24. https://doi.org/10.1007/978-3-319-90656-0_1

10. Tripathi V, Tripathi P. Antibiotic resistance genes: An emerging environmental polluant. Environ Sci Eng (Subser Environ Sci). 2017;(9783319462479):iii–iv:183–201

11. Agaba P, Tumukunde J, Tindimwebwa JVB, Kwizera A. Nosocomial bacterial infections and their antimicrobial susceptibility patterns among patients in Ugandan intensive care units: A cross sectional study. BMC Res Notes. 2017;10(1):349

12. Fernando SA, Gray TJ, Gottlieb T. Healthcare-acquired infections: Prevention strategies. Intern Med J. 2017;47(12):1341–51

13. Huang SS, Datta R, Platt R. Risk of acquiring antibiotic-resistant bacteria from prior room occupants. Arch Intern Med. 2006;166(18):1945–51

14. Rello J, Kalwaje Eshwara V, Lagunes L, Alves J, Wunderink RG, Conway-Morris A, et al. A global priority list of the TOp TEn resistant microorganisms (TOTEM) study at intensive care: A prioritization exercise based on multi-criteria decision analysis. Eur J Clin Microbiol Infect Dis. 2019;38(2):319–23

15. Arias CA, Murray BE. The rise of the Enterococcus: Beyond vancomycin resistance. Nat Rev Microbiol. 2012;10(4):266–78

16. Blanc DS. The use of molecular typing for epidemiological surveillance and investigation of endemic nosocomial infections. Infect Genet Evol. 2004;4(3):193–7

17. Han JH, Sullivan N, Leas BF, Pegues DA, Kaczmarek JL, Umscheid CA. Cleaning Hospital room surface to prevent health care-associated infections. Ann Intern Med. 2015;163(8):598–607

18. BrCAST. Orientações do EUCAST para a detecção de mecanismos de resistência e resistências específicas de importância clínica e/ou epidemiológica. Retrieved from: http://brcast.org.br/documentos/;46; 2018

19. Brasil. Portaria N° 64, de 11 de dezembro de 2018 [Internet]; 2018. Retrieved from: https://www.inca.gov.br/tipos-de-cancer/cancer-anal. p. 1

20. Snoad C, Nagel C, Bhattacharya A, Thomas E. The Effectiveness of Sanitary Inspections as a Risk Assessment Tool for Thermotolerant Coliform Bacteria Contamination of Rural Drinking Water: A Review of Data from West Bengal, India. Am J Trop Med Hyg. 2017 Apr;96(4):976-983. doi: 10.4269/ajtmh.16-0322. Epub 2017 Jan 23. PMID: 28115676; PMCID: PMC5392651.

21. Chemaly RF, Simmons S, Dale C, Ghantoji SS, Rodriguez M, Gubb J et al. The role of the healthcare environment in the spread of multidrug-resistant organisms: Update on current best practices for containment. Ther Adv Infect Dis. 2014;2(3–4):79–90

22. Chaoui L, Mhand R, Mellouki F, Rhallabi N. Contamination of the surfaces of a health care environment by multidrug-resistant (MDR) bacteria. Int J Microbiol. 2019;2019:3236526

23. Gray JW, Mahida N. How do you solve a problem like multidrug-resistant Gram-negative bacteria? J Hosp Infect. 2016;92(1):1–2. http://dx.doi.org/10.1016/j.jhin.2015.11.005

24. Kramer A, Schwebke I, Kampf G. How long do nosocomial pathogens persist on inanimate surfaces? A systematic review. BMC Infect Dis. 2006;6:1–8

25. Molter G, Seifert H, Mandraka F, Kasper G, Weidmann B, Hornei B, et al. Outbreak of carbapenem-resistant Acinetobacter baumannii in the intensive care unit: A multi-level strategic management approach. J Hosp Infect. 2016;92(2):194–8. http://dx.doi.org/10.1016/j.jhin.2015.11.007

26. Köhler AT, Rodloff AC, Labahn M, Reinhardt M, Truyen U, Speck S. Evaluation of disinfectant efficacy against multidrug-resistant bacteria: A comprehensive analysis of different methods. Am J Infect Control. 2019;47(10):1181–7

27. Ikeda T, Ledwith A, Bamford CH, Hann RA. Interaction of a polymeric biguanide biocide with phospholipid membranes. Biochim Biophys Acta. 1984 Jan 11;769(1):57-66. doi: 10.1016/0005-2736(84)90009-9. PMID: 6691980.

29. Tondo EC, Machado TRM, Malheiros Pda S, Padrão DK, de Carvalho AL, Brandelli A. Adhesion and biocides inactivation of Salmonella on stainless steel and polyethylene. Braz J Microbiol. 2010;41(4):1027–37

30. Wessels S, Ingmer H. Modes of action of three disinfectant active substances: A review. Regul Toxicol Pharmacol. 2013;67(3):456–67. http://dx.doi.org/10.1016/j.yrtph.2013.09.006

31. Mulani MS, Kamble EE, Kumkar SN, Tawre MS, Pardesi KR. Emerging strategies to combat ESKAPE pathogens in the era of antimicrobial resistance: A review. Front Microbiol. 2019;10(APR):539

32. De Oliveira DMP, Forde BM, Kidd TJ, Harris PNA, Schembri MA, Beatson SA, et al. Antimicrobial resistance in ESKAPE pathogens. Clin Microbiol Rev. 2020;33(3):1–49

33. Santajit S, Indrawattana N. Mechanisms of antimicrobial resistance in Pasteurellaceae. Pbiomed Res Int. 2016;2016(1155):1–8

34. Weber KL, Lesassier DS, Kappell AD, Schulte KQ, Westfall N, Albright NC, et al. Simulating transmission of ESKAPE pathogens plus C. difficile in relevant clinical scenarios. BMC Infect Dis. 2020;20(1):1–15

35. Ma YX, Wang CY, Li YY, Li J, Wan QQ, Chen JH, et al. Considerations and caveats in combating ESKAPE pathogens against nosocomial infections. Adv Sci (Weinh). 2020;7(1):1901872

36. Abbas A, Srivastava P, Nirwan PS. Prevalence of MLSB resistance and observation of erm A & erm C Genes at a tertiary care hospital. J Clin Diagn Res. 2015;9(6):DC08–DC10

37. Yahav D, Giske CG, Grāmatniece A, Abodakpi H, Tam VH, Leibovici L. New β-lactam–β-lactamase inhibitor combinations. Clin Microbiol Rev. 2020;34(1):1–61

38. Tsitou VM, Mitov I, Gergova R. Relationship between MLSB resistance and the prevalent virulence genotypes among Bulgarian Staphylococcus aureus isolates. Acta Microbiol Immunol Hung. 2020;68(1):55–61

39. Cerceo E, Deitelzweig SB, Sherman BM, Amin AN. Multidrug-resistant Gram-negative bacterial infections in the hospital setting: Overview, implications for clinical practice, and emerging treatment options. Microb Drug Resist. 2016;22(5):412–31

40. Walger P. Rationaler Einsatz von Antibiotika. Internist. 2016;57(6):551–68

41. Wille I, Mayr A, Kreidl P, Brühwasser C, Hinterberger G, Fritz A, et al. Cross-sectional point prevalence survey to study the environmental contamination of nosocomial pathogens in intensive care units under real-life conditions. J Hosp Infect. 2018;98(1):90–5. https://doi.org/10.1016/j.jhin.2017.09.019

42. Vermeil T, Peters A, Kilpatrick C, Pires D, Allegranzi B, Pittet D. Hand hygiene in hospitals: Anatomy of a revolution. J Hosp Infect. 2019;101(4):383–92. https://doi.org/10.1016/j.jhin.2018.09.003

43. Tenover FC. Mechanisms of antimicrobial resistance in bacteria. Am J Infect Control. 2006;34(5)(suppl. 1):S3–10; discussion S64

44. Drees M, Snydman DR, Schmid CH, Barefoot L, Hansjosten K, Vue PM, et al. Prior environmental contamination increases the risk of acquisition of vancomycin-resistant enterococci. Clin Infect Dis. 2008;46(5):678–85

45. Hardy KJ, Gossain S, Henderson N, Drugan C, Oppenheim BA, Gao F, et al. Rapid recontamination with MRSA of the environment of an intensive care unit after decontamination with hydrogen peroxide vapour. J Hosp Infect. 2007;66(4):360–8

46. Ortega Morente E, Fernández-Fuentes MA, Grande Burgos MJ, Abriouel H, Pérez Pulido R, Gálvez A. Biocide tolerance in bacteria. Int J Food Microbiol. 2013;162(1):13–25. http://dx.doi.org/10.1016/j.ijfoodmicro.2012.12.028

47. Gerba CP. Quaternary ammonium biocides: Efficacy in application. Appl Environ Microbiol. 2015;81(2):464–9.

Tables

Table 1. Risk analysis of contamination by bacterial isolates in the intensive care unit (ICU) environment.

ICU locations	ICU surfaces	R_{bd}	Risk level	R_{ad}	Risk level	R_{tt}	Risk level	$Fold_{risk}$	S
Administrative	**Total**	**0.2**	**I**	**2.0**	**II**	**2.2**	**II**	**10.0**	+
	Floor	0.2	I	2.0	II	2.2	II	10.0	+
Bathroom	**Total**	**0.5**	**I**	**2.2**	**II**	**2.7**	**III**	**4.4**	+
	Tile	0.3	I	2.0	II	2.3	II	6.7	+
	Floor	0.2	I	0.2	I	0.4	I	1.0	#
Pantry	**Total**	**2.2**	**II**	**4.8**	**III**	**7.0**	**IV**	**2.2**	++
	Floor	1.2	II	4.4	III	5.6	IV	3.7	+
	Microwave	0.5	I	0.2	I	0.7	I	0.4	#
	Fridge	0.5	I	0.2	I	0.7	I	0.4	#
Pharmacy	**Total**	**1.7**	**II**	**4.4**	**III**	**6.1**	**IV**	**2.6**	+
	Floor	1.2	II	2.0	II	3.2	III	1.7	#
	Stand	0.3	I	0.4	I	0.7	I	1.3	#
	Glass	0.2	I	2.0	II	2.2	II	10.0	+
Beds	**Total**	**1.5**	**II**	**26.8**	**V**	**28.3**	**V**	**17.9**	+++
	Bed	0.5	I	2.8	III	3.3	III	5.6	+++
	Floor	0.3	I	10.8	V	11.1	V	36	++++
	Table	0.3	I	2.8	III	3.1	III	9.3	++
	Cardiac monitor	0.4	I	10.4	V	10.8	V	26.0	++++
Sink	**Total**	**0.3**	**I**	**0.4**	**I**	**0.7**	**I**	**1.3**	#
	Sink	0.3	I	0.4	I	0.7	I	1.3	#

R_{bd} - risk before disinfection; R_{ad} - risk after disinfection; R_{tt} - total risk; $Fold_{risk}$ - real risk observed; S - significance; + increase in 1 risk level; ++ increase in 2 risk levels; +++ increase in 3 risk levels; ++++ increase in 4 risk levels; # no change in risk level; level I = very low; level II = low; level III = intermediate; level IV = high; and level V = very high.
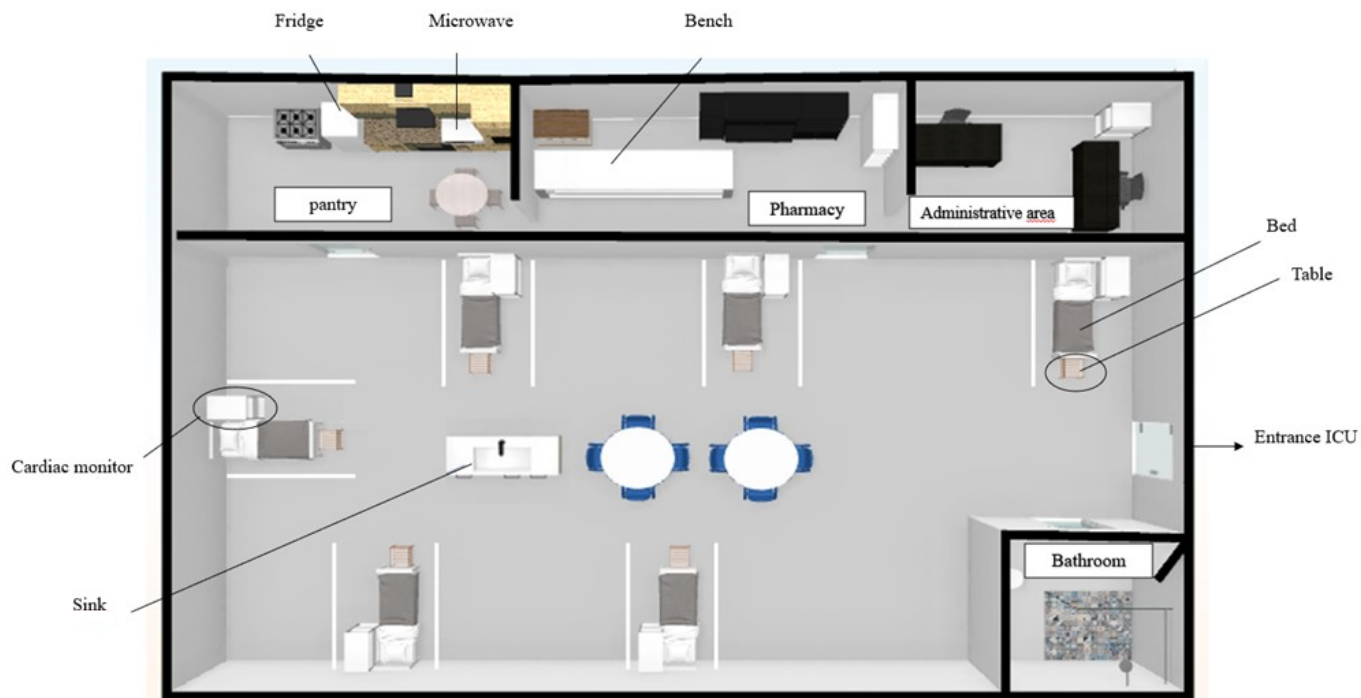
Figures

Figure 1

Intensive care unit (ICU) area and collection sites scheme. The ICU settings and surfaces identified included the administrative area, bathroom, pantry, pharmacy, beds, and sink.
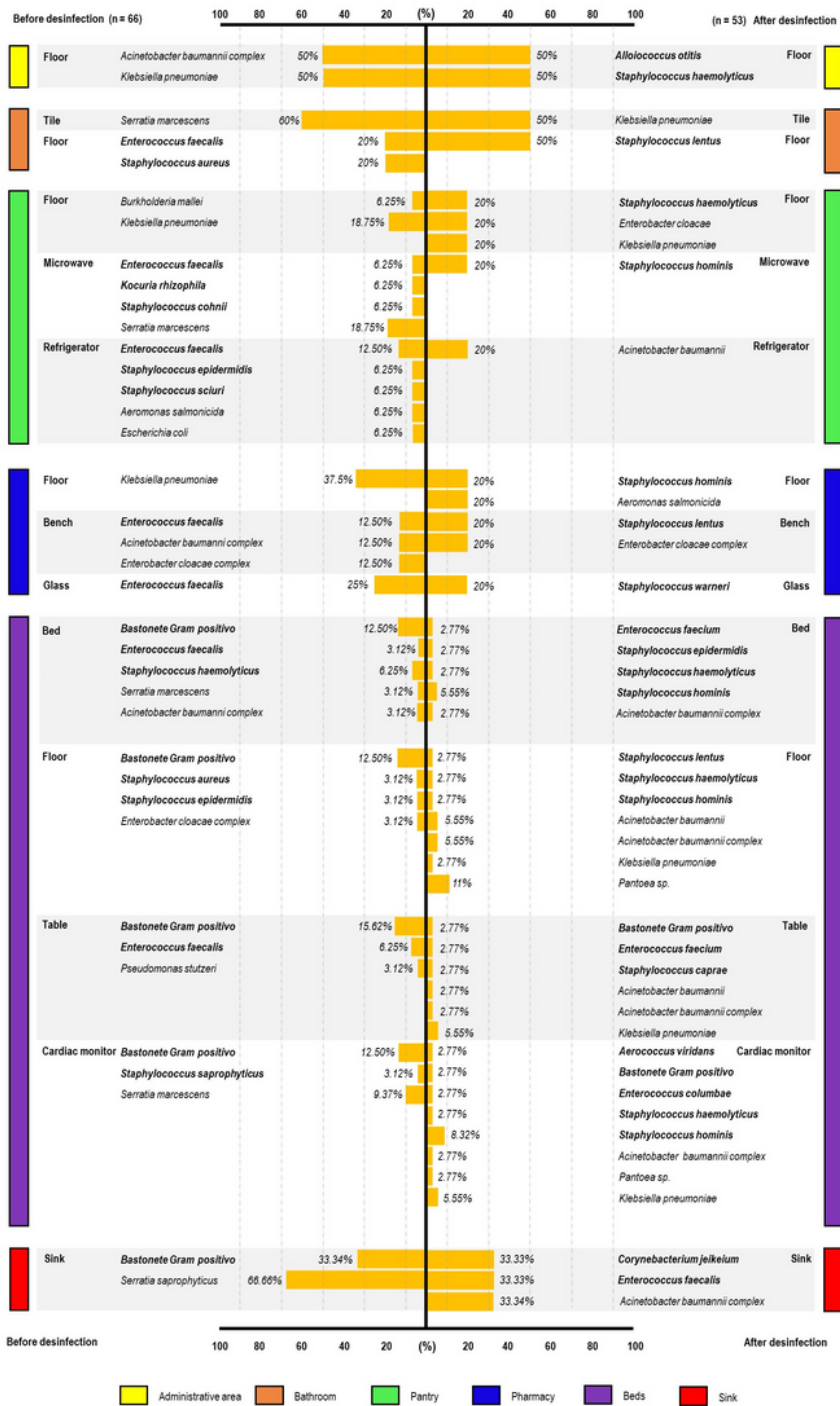
Figure 2

Frequency distribution of the bacterial strains isolated from the ICU setting. Data are expressed as the percentage of the strain isolated before (left) and after (right) the disinfection process. The ICU settings and surfaces were identified by colors: yellow - administrative area; orange - bathroom; green - pantry; blue - pharmacy; purple - beds; and red - sink.
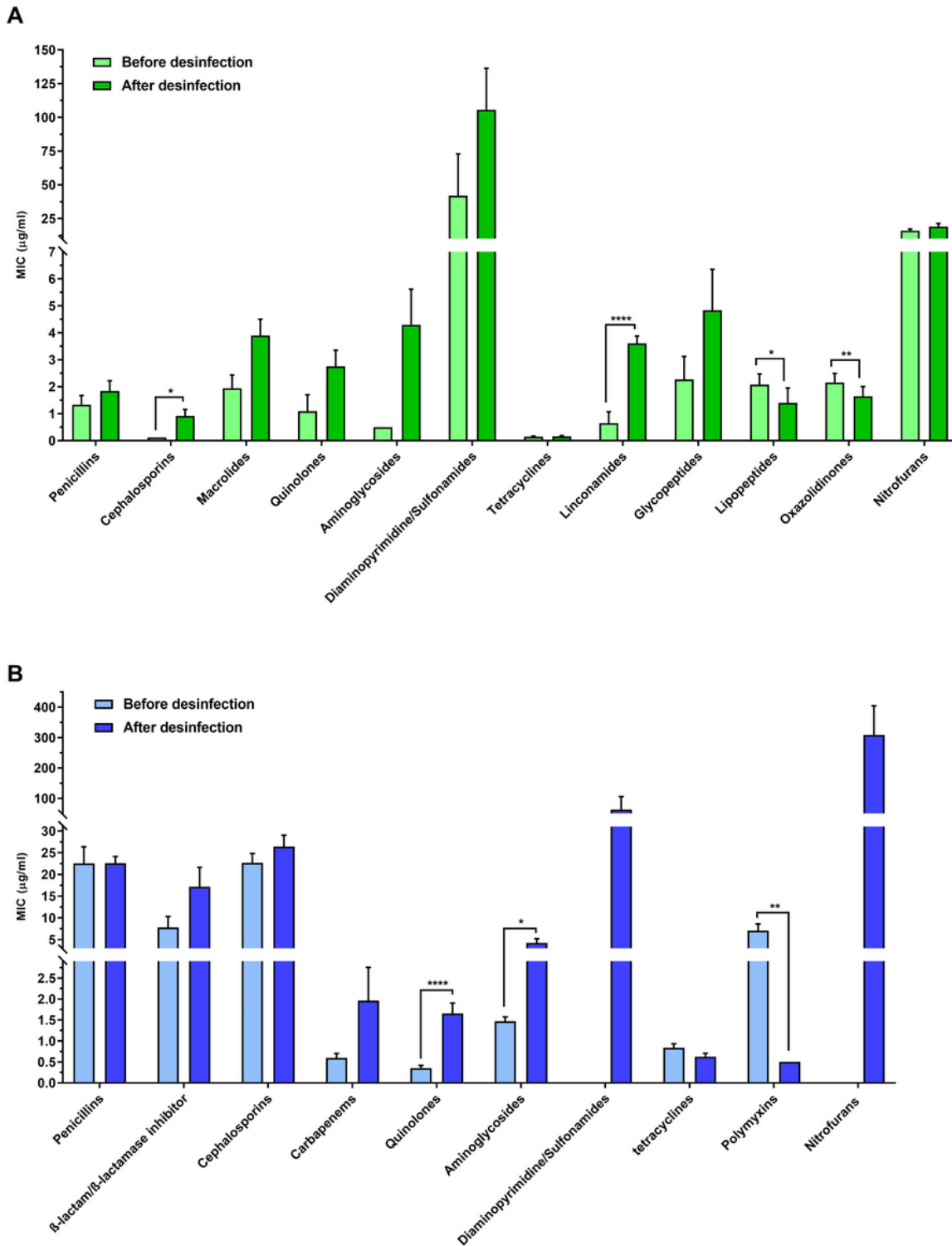
Figure 3

Comparison of the minimum inhibitory concentration (MIC) values of different classes of antimicrobials used against bacterial isolates from a nosocomial environment, before and after disinfection in the ICU setting. (A) Gram-positive isolates and (B) Gram-negative isolates. Mann-Whitney test. Data are represented as the mean ± standard error of the mean (SEM). Values of $p < 0.05$ were considered to be statistically significant. * $p < 0.05$; ** $p < 0.01$; **** $p < 0.0001$.
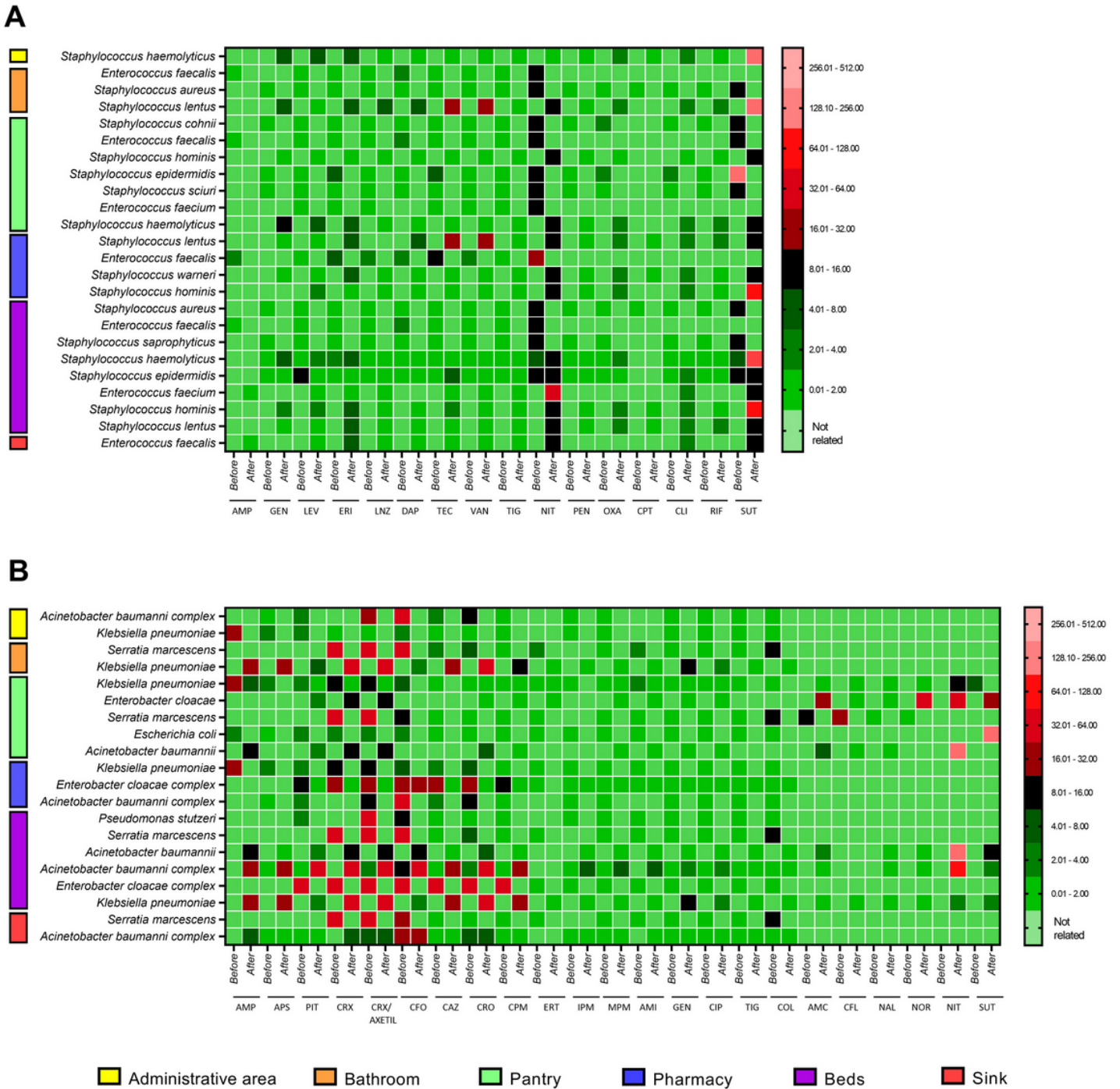
Figure 4

Heatmap of the antimicrobial susceptibility of bacteria, before and after the disinfection process in the ICU setting. The plotted MIC values represent the mean of each strain, before and after the disinfection of the ICU environment. The strains are grouped according to the location of isolation in the ICU: administrative area (yellow), bathroom (orange), pantry (green), pharmacy (blue), beds (purple), and sink (red). (A) Comparison of MICs for gram-positive isolates and (B) gram-negative isolates. Amoxicillin/clavulanic acid (AMC); Amikacin (AMI); Ampicillin (AMP); Ampicillin/Sulbactam (APS); Ceftazidime (CAZ); Cephalothin (CFL); Cefoxitin (CFO); Ciprofloxacin (CIP); Clindamycin (CLI); Colistin

(COL); Cefepime (CPM); Ceftaroline (CPT); Ceftriaxone (CRO); Cefuroxime (CRX); Cefuroxime/Axetil (CRX/AXETIL); Daptomycin (DAP); Erythromycin (ERI); Ertapenem (ERT); Gentamicin (GEN); Imipenem (IPM); Levofloxacin (LEV); Linezolid (LNZ); Meropenem (MPM); Nalidixic acid (NAL); Nitrofurantoin (NIT); Norfloxacin (NOR); Oxacillin (OXA); Benzylpenicillin (PEN); Piperacillin/Tazobactam (PIT); Rifampicin (RIF); Trimethoprim/Sulfamethoxazole (SUT); Teicoplanin (TEC); Tigecycline (TIG); Vancomycin (VAN).
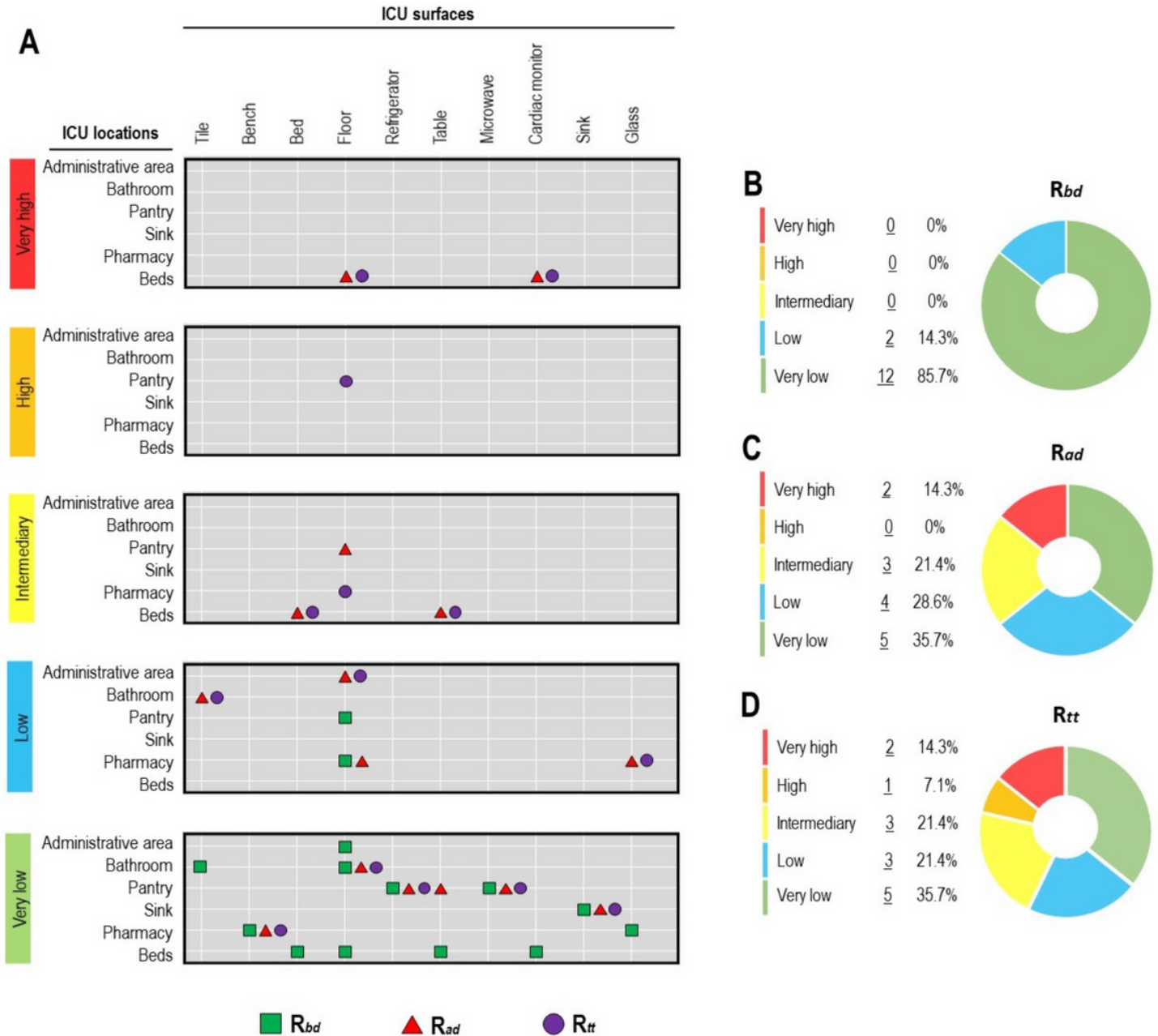

Figure 5

Risk analysis of bacterial contamination in the ICU setting. (A) The risk before disinfection (Rbd - green square), after disinfection (Rad - red triangle), and the total risk (Rtt - purple circle) of contamination for each surface at the respective ICU locations were calculated and grouped according to risk levels: very low (green), low (blue), intermediate (yellow), high (orange), and very high (red), on the left. Frequency

distribution of the risk (B) before disinfection (Rbd), (C) after disinfection (Rad), and (D) total risk (Rtt) in the ICU analyzed.
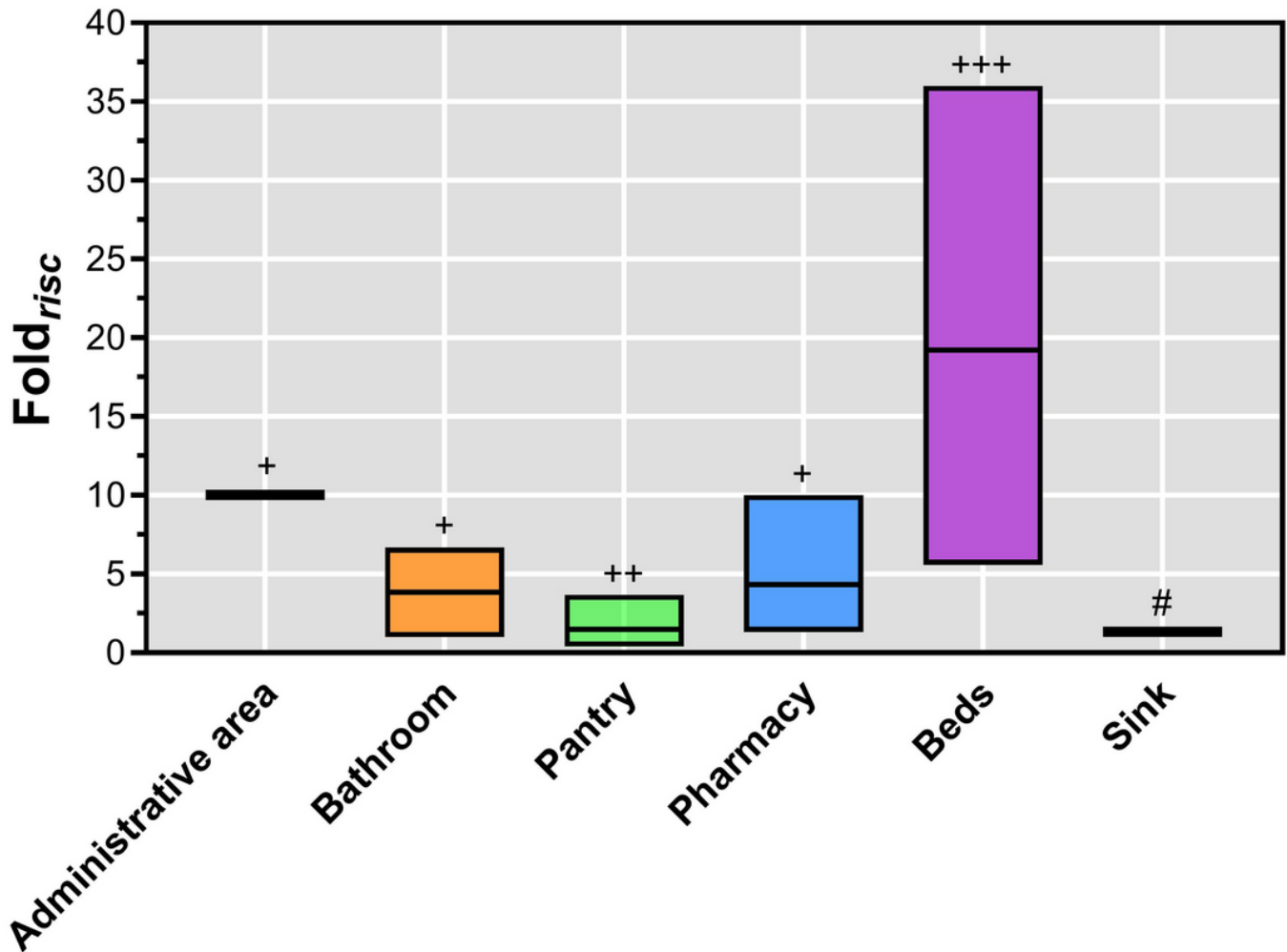

Figure 6

Disinfection process increases the real risk of contamination by bacterial isolates in the ICU setting. The adjusted real risk observed values (Foldrisk) assess whether the calculated real risk observed resulted in a change in the risk level at different locations in the ICU. Data are presented as floating bars with mean ± min to max values. +increase in 1 risk level; ++ increase in 2 risk levels; +++ increase in 3 risk levels; ++++ increase in 4 risk levels; # no change in risk level.