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Microbial Load of Hand Sanitizer Dispensers—A University Hospital Study

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Abstract: Hospital-acquired infections are a significant concern in healthcare settings, leading to patient safety risks, increased morbidity and mortality, and financial burdens. Hand hygiene is crucial in preventing the spread of bacteria in hospitals and communities. Manual hand sanitizer dispensers can harbor presumptive pathogenic bacteria and act as fomites for bacterial transmission. This study aimed to assess the microbial contamination of manual hand sanitizer dispensers in a hospital setting and to study their antibiotic resistance profiles. Samples were collected using sterile cotton swabs and then inoculated into brain heart infusion broth. Subsequent subcultures were performed on both blood and MacConkey agar. The isolates were then identified using the Bruker MALDI Biotyper (Bruker Daltonik, Bremen, Germany) to the species level. Sampling was conducted in various wards and in the hospital and the University areas on dispenser levers and nozzle areas. The results showed that all samples yielded one or more bacterial species. Bacterial isolates identified belonged to species commonly found on the skin microflora and some Gram-negative enteric bacilli. Higher colonization was observed on the dispenser lever. Among Gram$^+$ microorganisms, most bacterial species were shown to be sensitive to β-lactams, with the exception of *Staphylococcus* spp., resistant to AMP (Ampicillin) and Penicillin. However, no Methicillin resistant isolates were detected. Gram$^-$ microorganisms such as *Pseudomonas luteola* were shown to be sensitive to all tested antibiotics, while *Pantoea agglomerans* was shown to be resistant to AMC (amoxicillin–clavulanic acid). Rifampicin tested only against Bacilli showed resistance. Based on the findings, it is recommended to implement systematic cleaning and proper maintenance of manual dispenser areas or to use automated dispensers to reduce hand contact and minimize microbial contamination. Monitoring the presence of microorganisms in hand sanitizing gels and dispensers is an essential infection control strategy.

Keywords: hospital-acquired infections; hand hygiene; hand sanitizer dispensers; microbial contamination; MALDI-TOF MS; alcohol-based hand sanitizer; COVID-19; patient safety; antibiotic sensitivity test (AST)

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1. Introduction

The percentage of infections related to medical practices in the hospital and between patients and medical staff varies between 4 and 9%, adding an extra burden on the health system by escalated morbidity, mortality and hospitalization costs [1–3]. Due to the importance of hand disinfection among healthcare workers and patients, in the treatment of hospital-acquired infections (HAIs) and in the development of multi-resistant pathogens, specific and detailed instructions and guidelines for hand hygiene have been issued by

international organizations since the 2000s [4,5]. One of the most effective ways to reduce hospital-acquired infections and communicable diseases within the hospital environment is to disinfect hands with alcohol-based hand sanitizers [6,7].

The COVID-19 pandemic crisis has changed many things regarding hygiene and the public perceptions of hand hygiene and simple and practical methods of hand disinfection. Following the recommendations of international organizations and individual state initiatives, one of the measures to avoid the spread of COVID-19 was, among others, the use of hand sanitizers, mainly alcohol-based. Their use spread not only in hospital areas but also in restaurants, supermarkets and people's daily lives [8]. Their ease of use has made alcohol-based hand sanitizers very popular, driving their global production to tremendous growth. Typical specifications for commercial hand sanitizers include the alcohol type (n-propyl alcohol, isopropyl alcohol, ethanol or a combination of them) and a minimum of 60% alcohol to achieve their disinfecting action properly [9,10].

In addition, hand hygiene is also an important part of hospital hygiene planning that prevents the further transmission of diseases and antimicrobial microorganisms [11]. The use of hand sanitizers placed on wards, at the entrance of clinics and around the patient's bed was also used before the COVID pandemic. An important link in the chain of hygiene within the hospital premises and the hospital environment is the workers in them. The frequency of using alcohol-based hand rubs among workers is of the utmost importance, and various ways of measuring this hand hygiene practice have been proposed, such as the time of their placement on the hospital premises and the measurement of their consumption [12,13].

The importance of hand hygiene among hospital workers has also been recognized by the World Health Organization, resulting in the development of a methodological framework for hand disinfection at various stages of hospital patient care, as well as training and follow-up plans of practices [13]. This framework presupposes the disinfection of the hands of hospital workers as an imperative practice, fully integrated into the daily workflow, with awareness of the risk of the spread of multi-susceptible microorganisms in the hospital environment [11]. The practical application of this program was called "My five moments for hand hygiene" and includes hand disinfection and hygiene regarding patient treatment, such as before patient contact or before implementing an aseptic task, after body fluid exposure and after contact with the patient and finally after contact with patient surroundings [11–13]. Also, compliance with the hand hygiene of the hospital staff does not only concern the bed and the area around the patients in their bed, but the additional practices when moving them to other areas of the hospital for diagnostic tests, as well as their transport to the hospital with any vehicle, where hand hygiene also plays an important role in preventing infections [14].

Hand sanitizers in a hospital must be easily accessible and constantly available to reduce hospital-acquired infections. Spatial location within a hospital is a critical issue for staff utilization. Factors such as the ease of access, the height of the installation on the wall and visibility in patient rooms, at the entrances of special clinics, such as post-surgery rooms, and in waiting areas are among other factors that must be taken into account for the placement and access to hand sanitizers in a hospital environment [12,15]. The 'accessibility' factor of hand sanitizers has been highlighted as one of the least important, with the World Health Organization specifying that hand sanitizers should be available in-patient rooms without specific numerical criteria for their density [5]. Countries such as Germany and Sweden have promoted specific guidelines for the number of hand sanitizers per patient bed to at least 0.5 per patient bed for Germany and 2.4 for Sweden, taking into account the hospital type and bed count [16,17].

Hand sanitizers should be available in all hospital wards and areas, and their use by all patients, visitors and hospital staff should be encouraged. The latter should be educated on the correct use of hand sanitizers, which should happen during nursing practice. Also, the nursing and medical staff should be reminded to act as behavioral models regarding hand hygiene because the spread of multi-resistant bacteria and the increase in nosocomial

infections partly depend on their compliance [18]. Hand disinfection and its importance in reducing nosocomial infections have resulted in its being proposed as a standard and horizontal practice to minimize the spread of resistant microorganisms, compared to other classic methods, such as using soap and water, which require time and extra costs [16,17]. Combined with an additional network of actions such as staff training and automated wall hand sanitizers to monitor and assess staff compliance, staff and patient [19,20] hand disinfection can be an essential tool against nosocomial infections and protect the health of patients and workers [20].

Although there have been studies concerning the antimicrobial action of hand sanitizers on various microorganisms and from different alcoholic components and combinations, it is impossible to simulate all the realistic hygienic conditions in a hospital [8,21]. Also, tests of the virucidal action of hand sanitizers have shown that the activity of alcoholic antiseptics against specific viruses requires a particular time to achieve their action, e.g., 50 and 70% ethanol reduced the infectivity of feline calicivirus by $\geq$2.2 log units after exposure for five minutes. Also, increased concentrations of alcohol result in its rapid evaporation from the container and perhaps in reduced effectiveness against some viruses and pathogenic microorganisms. Therefore, the action of hand sanitizers depends on the frequency of their use and the composition and alcohol content, so that the containers and hand sanitizers cannot harbor pathogenic bacteria or viruses and contribute to their further transmission in the hospital environment [22–25].

This research aims to investigate the presence of bacterial species on hand sanitizers and to determine the antimicrobial resistance traits that can disseminate within the hospital environment while underscoring the critical significance of these findings for public health. Thus, samples will be taken from dispenser levers and nozzles with cotton swabs in various clinics of a tertiary university hospital in Thrace, Northern Greece, which provides health facilities to a region of about 350,000 inhabitants, and in departments of the medical school, in order to identify the microbial flora hosted by hand sanitizers in various hospital and university areas. The sanitizer dispensers in the hospital will be situated in areas that hospital personnel, patients and visitors have access to. Finally, the possibility of a correlation between specific pathogenic bacteria in the hospital and medical school areas will be evaluated.

2. Materials and Methods

Sampling Procedure

Surface samples were collected from dispensers located in various areas of the hospital and the campus that were available to the public, including hospital wards and clinics, cafeterias, classrooms, etc. Samples were collected from sanitizer dispenser levers and nozzles with cotton swabs and transferred to the laboratory for further investigation. Sample size was assessed according to the accessibility of the hand sanitizer dispensers. Every sample station consisted of up to 3 dispensers. A total of 50 samples were collected from an equivalent number of dispensers in June 2022. After scrubbing the surface of the sanitizer's dispenser 10 times, swabs were inoculated into brain heart infusion broth and incubated in 37 °C for 24 h. Twenty-four hours later, subcultures were streaked on both Blood and MacConkey agar following common procedures (Figure 1).

Due to COVID-19 regulation policies, access to most clinics was restricted to patients and healthcare staff. All available dispensers from the University Hospital, as well as from the Department of Medicine in the University were tested. Consequently, only 50 sampling stations were available to enter this research study and the results were evaluated accordingly.

Various antibiotics representing different classes commonly employed in clinical practice were chosen. This selection included antimicrobial agents for Gram-negative bacteria, for Gram-positive bacteria and for *Bacillus* spp.

Pure cultures were identified using Microflex LT MALDI-TOF MS (Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry/Bruker Daltonics, Bremen,

Germany). Five single colonies were taken directly from the nutrient agar plate and the sample preparation was performed according to the instructions of Bruker Daltonics and analyzed by running FlexControl 3.4 software. Each sample was spotted in triplicate. For each sample, the MS signals were acquired in a positive linear manner, according to the manufacture's instructions. The obtained experimental MALDI-TOF mass spectrum profiles were then compared to the reference spectra and the matching between them was expressed by a Biotyper Log (score). The Log (score) considered the number of matching peaks, the total number of peaks, the peak weight representing species specificity and a correlation factor related to the matching peak intensity. All samples were measured on a mass spectrometer (Bruker Daltonics, Germany) using the method "MBT_AutoX" and the peaks were evaluated with the processing method "MBT_Process" using FlexControl (version 3.4, Bruker Daltonics, Germany). A calibration took place by using the Bacterial Test Standard (BTS) from Bruker. All spectra were compared with reference spectra of the BDAL database (version 8.0). Scores lower than 1.700 were interpreted as having no identification, while scores up to 2.000 can be considered species-level identification.

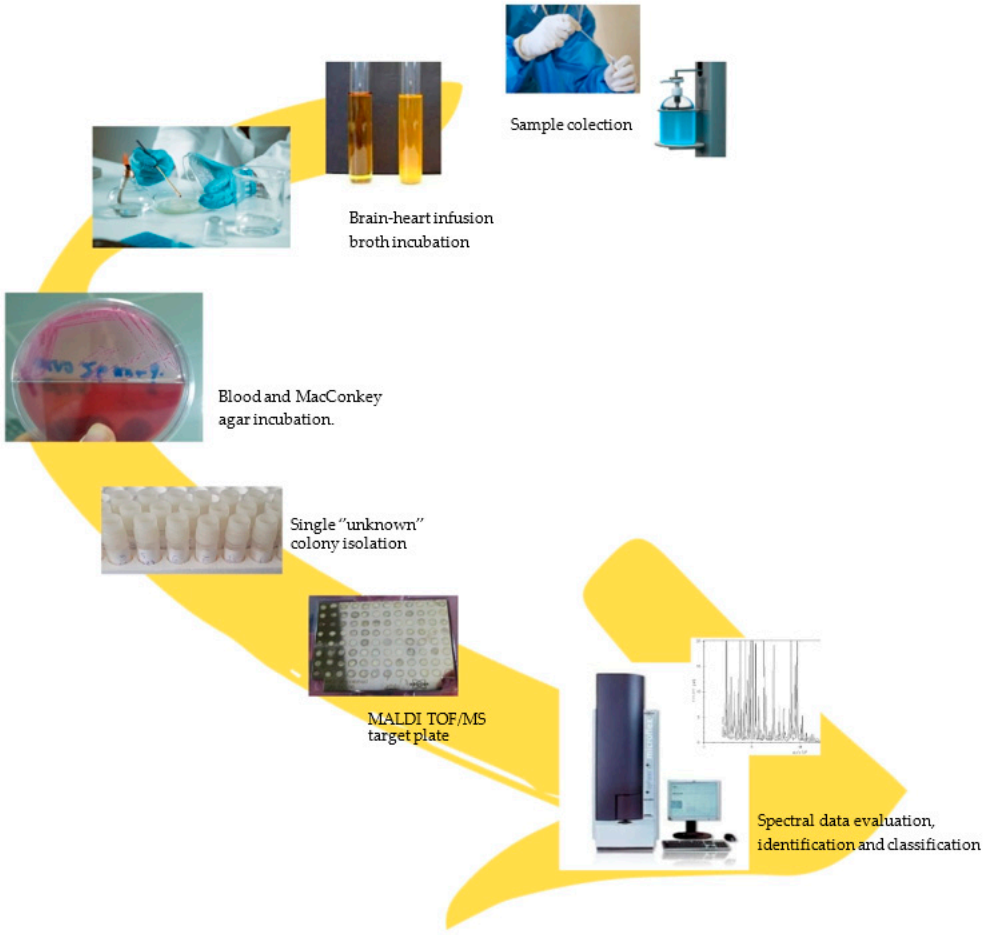

Figure 1. Study design: general workflow of bacteria collection, growth, isolation, identification and classification with MALDI-TOF-MS.

The assessment of antimicrobial resistance profiles in the isolated bacteria was conducted using the agar disk diffusion method, following the criteria established by the National Committee for Clinical Laboratory Standards (NCCLS) [26].

3. Results

A total of 27 isolates of bacterial species were identified in 22 out of 50 sampling stations. As shown in Figure 2, the distribution of samples from hospital and university was 80% and 20%, respectively. The most dominant bacterial species identified belong

to the genus of *Staphylococcus*, comprising 55.5% of the total according to MALDI-TOF analysis. It is worth mentioning that the count of isolated species, and subsequently the number of identified ones, was notably lower than anticipated considering our sample size. Since the sampling from the dispensers occurred at random time intervals throughout the day, this outcome can likely be attributed to the thorough cleaning that might have been performed on the dispensers by the cleaning staff in various areas of the sampling process. Moreover, due to the COVID-19 pandemic, the extensive use of hand sanitizers in everyday routine might contribute to the low percentage of devices' contamination.

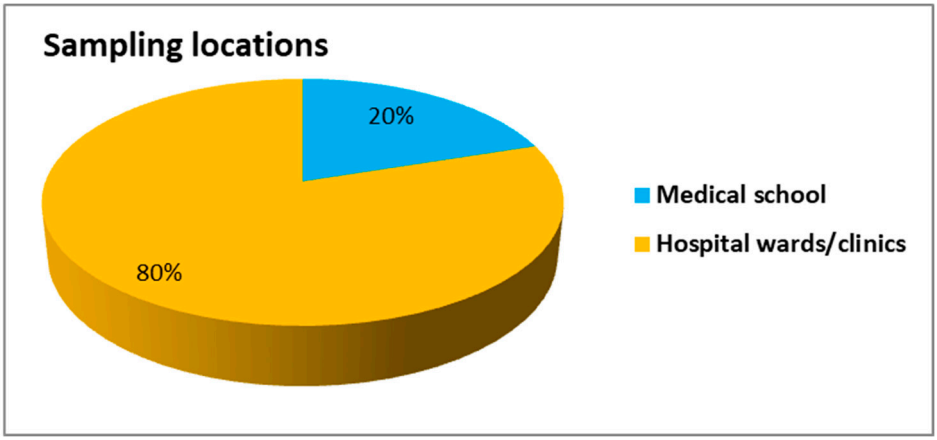

Figure 2. Distribution of sampling locations.

In the Neurosurgery ward, only *Staphylococcus cohnii* was identified, with a scoring value of 2.32, interpreted as a high-confidence identification. In all maternity ward samples, the identified bacterial isolates included *Staphylococcus warneri* with a score value of 2.04, *Staphylococcus epidermidis* with a score value of 2.19, *Bacillus megaterium* with a score value of 2.20, *S. epidermidis* with a score value of 2.07 and *Staphylococcus hominis* with a score value of 2.36. By the main hospital entrance, five species were identified, in particular *Solibacillussilvestris* with a score value of 1.73 (low confidence identification), while *Staphylococcus caprae*, *S. epidermidis*, *Micrococcus luteus* and *Pseudomonas luteola* showed high score values: 2.05, 2.13, 2.44 and 2.33, respectively. By the chemotherapy ward, *Staphylococcus aureus* and *Pantoea agglomerans* were identified with score values of 2.31 and 2.32, respectively.

Furthermore, from all outpatient clinic samples a total of six species were identified, and they were *S. caprae* with a score value of 2.21, *Staphylococcus lugdunensis* with a score value of 2.22, *S. epidermidis* with a score value of 2.14, *Mixta calida* with a score value of 1.80, *Staphylococcus haemolyticus* with a score value of 2.03 and *Staphylococcus saprophyticus* with a score value of 2.10. In the waiting room of the CAT scan department of the hospital, *Lysinibacillus xylanilyticus* was identified with a score value of 2.02. In postoperative care, only one of the two bacterial species were identified, namely *Bacillus cereus* with a score value of 2.03. In the neurological clinic, no bacteria were identified.

In the university area, three different sample stations yielded four identified bacterial isolates. On the dispenser by the cafeteria, *B. megaterium* was identified with a score value of 2.21; by the histology department, *Cytobacillus oceanisediminis* with a score value of 2.00; and by the hygiene department, *Paenibacillus amyloliquefaciens* and *Staphylococcus xylosus* with score values of 2.00 and 1.82, respectively. Table 1, below, summarizes all findings in three columns that include the sample location, the bacterial species identification and the score value calculated through MALDI-TOF.

In total, 27 isolates were processed for identification by MALDI-TOF. Twenty-three of them were identified to the species level, with a score of up to 2.00, three of them were identified in a low-confidence level, and one was not identified.

All Gram+ bacteria which were isolated from the dispensers and had been identified as previously mentioned were tested against the following thirteen (13) antimicro-

bial agents: Chloramphenicol (CHL—30 mcg), Ciprofloxacin (CIP—5 mcg), Gentamycin (GEN—10 mcg), Erythromycin (ERY—15 mcg), Trimethoprim/sulfamethoxazole (SXT—1.25 + 23.75 mcg), Tetracycline (TET—30 mcg), Amoxicillin–Clavulanic (AMC—20/10 mcg), Cefoxitin (CFX—30 mcg), Oxacillin (CXI—1 mcg), Ampicillin (AMP—10 mcg), Penicillin G (PIP—10 mcg), Vancomycin (VAN—30 mcg) and Rifampicin (RIF—5 mcg) (Table 2).

Table 1. MALDI-TOF MS identification of the isolates from different sampling locations.

Sample Origin	Bacterial Species Best Match (MALDI-TOF)	Score Value [1]
Neurosurgery ward (corridor/entrance)	*Staphylococcus cohnii*	2.32
Maternity ward I (corridor/entrance)	*Staphylococcus warneri*	2.04
Maternity ward II (corridor/entrance)	*Staphylococcus epidermidis*, *Bacillus megaterium*, *Staphylococcus epidermidis*	2.19 2.20 2.07
Maternity ward III (corridor/entrance)	*Staphylococcus hominis*	2.36
Maternity ward IV (corridor/entrance)	*Staphylococcus hominis*	2.37
Main hospital entrance I	*Solibacillus silvestris*	1.73
Main hospital entrance II	*Staphylococcus caprae*	2.05
Main hospital entrance III	*Staphylococcus epidermidis*, *Micrococcus luteus*	2.13 2.44
Main hospital entrance IV	*Pseudomonas luteola*	2.33
Chemotherapy ward (waiting room)	*Staphylococcus aureus*, *Pantoea agglomerans*	2.31 2.32
Outpatient clinics (entrance)	*Staphylococcus caprae*	2.21
Outpatient clinics (surgeries)	*Staphylococcus lugdunensis* *Staphylococcus epidermidis*	2.22 2.14
Outpatient clinics (waiting room)	*Mixta calida*	1.80
Outpatient clinics (renal clinic waiting room)	*Staphylococcus haemolyticus*	2.03
Outpatient clinics (gynecology clinic waiting room)	*Staphylococcus saprophyticus*	2.10
CAT scan (waiting room)	*Lysinibacillus xylanilyticus*	2.02
Postoperative care (corridor/entrance)	*Bacillus cereus*	2.03
Postoperative care (info desk)	No organism identification possible	1.33
Neurological clinic (waiting room)	No organism identification possible	1.36
Cafeteria (University)	*Bacillus megaterium*	2.21
Dept. of Histology (University)	*Cytobacillus oceanisediminis*	2.00
Dept. of Hygiene (University)	*Paenibacillus amyloliquefaciens*, *Staphylococcus xylosus*	2.00 1.82

[1] Score value interpretation: 0.00–1.69: no organism identification possible; 1.70–1.99: low-confidence identification; 2.00–3.00: high-confidence identification.

Table 2. Antimicrobial resistance pattern of 13 antimicrobial agents against Gram(+) isolates from dispensers.

		Antimicrobial Agents												
	Class of Antibiotics	**Amphenicols**	**Fluoroquinolones**	**Aminoglycosides**	**Macrolide**	**Sulfonamides**	**Tetracyclines**		**β-Lactams**				**Glycopeptide**	**Rifampicin**
	Isolates	**CHL (30 mcg)**	**CIP (5 mcg)**	**GEN (10 mcg)**	**ERY (15 mcg)**	**TRS (1.25 + 23.75 mcg)**	**TET (30 mcg)**	**AMC (20/10 mcg)**	**CXI (30 mcg)**	**OXA (1 mcg)**	**AMP (10 mcg)**	**PIP (10 mcg)**	**VAN (30 mcg)**	**RIF (5 mcg)**
1	*Staphylococcus cohnii*	S	S	S	S	S	S	S	S	S	S	S	S	-
2	*Staphylococcus warneri*	S	S	S	S	S	S	S	S	S	S	S	S	-
3	*Staphylococcus epidermidis*	R	I	S	R	S	R	S	S	S	S	R	S	-
4	*Bacillus megaterium*	-	S	S	S	S	S	S	-	-	S	S	S	S
5	*Staphylococcus epidermidis*	S	S	S	S	S	S	S	S	S	S	R	S	-
6	*Staphylococcus hominis*	S	S	S	R	S	S	S	S	S	S	R	S	-
7	*Staphylococcus hominis*	S	S	S	R	S	S	S	S	S	S	R	S	-
8	*Staphylococcus caprae*	R	S	S	R	S	S	S	S	S	R	R	S	-
9	*Staphylococcus epidermidis*	S	S	S	R	S	R	S	S	S	I	R	S	-
10	*Micrococcus luteus*	S	S	S	S	S	S	S	-	-	S	S	S	-
11	*Staphylococcus aureus*	-	-	S	R	S	S	S	S	S	I	R	-	-
12	*Staphylococcus caprae*	S	S	S	R	S	S	S	S	S	S	R	S	-
13	*Staphylococcus lugdunensis*	S	S	S	R	S	S	S	S	S	S	S	S	-
14	*Staphylococcus epidermidis*	S	S	R	S	S	S	S	S	S	S	S	S	-
15	*Staphylococcus haemolyticus*	S	S	S	I	S	S	S	S	S	S	S	S	-
16	*Staphylococcus saprophyticus*	S	S	S	R	S	I	S	S	S	S	S	S	-
17	*Lysinibacillus xylanilyticus*	S	S	S	S	S	S	S	-	-	S	S	S	R
18	*Bacillus cereus*	S	S	S	S	I	S	R	-	-	R	R	S	R
19	*Bacillus megaterium*	S	S	S	S	S	S	S	-	-	S	S	S	R

Table 2. *Cont.*

		Antimicrobial Agents												
	Class of Antibiotics	**Amphenicols**	**Fluoroquinolones**	**Aminoglycosides**	**Macrolide**	**Sulfonamides**	**Tetracyclines**	**β-Lactams**					**Glycopeptide**	**Rifampicin**
	Isolates	**CHL (30 mcg)**	**CIP (5 mcg)**	**GEN (10 mcg)**	**ERY (15 mcg)**	**TRS (1.25 + 23.75 mcg)**	**TET (30 mcg)**	**AMC (20/10 mcg)**	**CXI (30 mcg)**	**OXA (1 mcg)**	**AMP (10 mcg)**	**PIP (10 mcg)**	**VAN (30 mcg)**	**RIF (5 mcg)**
20	*Cytobacillus oceanisediminis*	S	S	S	S	S	S	S	-	-	S	S	S	R
21	*Paenibacillus amyloliquefaciens*	S	S	S	S	S	S	S	-	-	S	S	S	R

Interpretation: S: sensitivity, R: resistance, I: intermediate, "-": did not perform, CHL: chloramphenicol, CIP: ciprofloxacin, GEN: gentamicin, ERY: erythromycin, TRS: trimethoprim/sulfamethoxazole, TET: tetracycline, AMC: amoxicillin–clavulanic, CXI: cefoxitin, OXA: oxacillin, AMP: ampicillin, PIP: penicillin G, VAN: vancomycin, RIF: rifampicin.

Table 3. Antimicrobial resistance pattern of 13 antimicrobial agents against Gram (-) isolates from dispensers.

		Antimicrobial Agents												
	Class of Antibiotics	**Amphenicols**	**Polymyxin**	**Fluroquinolones**		**Aminoglycosides**		**Sulfonamides**	**Tetracyclines**		**β-Lactams**			**Carbapenem**
	Isolates	**CHL (30 mcg)**	**COL (10 mcg)**	**CIP (5 mcg)**	**NAL (30 mcg)**	**GEN (10 mcg)**	**TOB (10 mcg)**	**SXT (1.25 + 23.75 mcg)**	**DOX (80 mcg)**	**TET (30 mcg)**	**AMC (20/10 mcg)**	**AMP (10 mcg)**	**IMI (10 mcg)**	**MER (10 mcg)**
22	*Pseudomonas luteola*	S	S	S	S	S	S	S	-	-	I	I	S	S
23	*Pantoea agglomerans*	S	S	S	S	S	S	S	-	-	R	S	S	S

Interpretation: S: sensitivity, R: resistance, I: intermediate, "-": did not perform, CHL: chloramphenicol, COL: colistin sulfate, CIP: ciprofloxacin, NAL: nalidixic acid, GEN: gentamicin, TOB: tobramycin, SXT: trimethoprim/sulfamethoxazole, DOX: doxycycline, TET: tetracycline, AMC: amoxicillin–clavulanic, AMP: ampicillin, IMI: imipenem, MER: meropenem.

Two isolates, one belonging to *S. epidermidis* species and one belonging to *S. caprae* species, have shown multi-drug resistance (resistant to more than three classes of antibiotics). The *Bacillus cereus* isolate was also found to be multi-resistant. Most of the isolates of the bacterial species tested were found to be susceptible to β-lactams, apart from *S. caprae*, resistant to AMP, and *S. aureus* and *S. epidermidis*, resistant to Penicillin. Oxacillin and Cefoxitin were tested only for *Staphylococcus* spp. isolates to determine if these strains were Methicillin resistant, but none of them were. Rifampicin was tested only against Bacilli, and all were found to be resistant. *Pseudomonas luteola* was found to be susceptible to all tested antibiotics, while *Pantoea agglomerans* was found to be resistant to AMC (Amoxicillin–Clavulanic acid) (Table 3). Twenty-three (23) of the isolates showed phenotypical resistance. None of the identified isolates belonging to the Staphylococci group, including both CNS and *S. aureus*, showed any resistance to Methicillin. In total, 33% of our CoNS isolates displayed multi-drug resistance.

4. Discussion

The challenge of antimicrobial resistance (AMR) in bacterial pathogens continues to be a significant concern in the field of infection control. To comprehensively address this issue, the One Health concept is actively being pursued. This approach acknowledges the equal importance of humans, domestic and wild animals and the environment, recognizing their interconnectedness. It also extends its scope to include commensal and environmental bacteria in order to evaluate the factors that contribute to the development of AMR in pathogens [27].

The coexistence of various microbial communities within shared ecological niches facilitates the broad distribution of genetically encoded resistance characteristics. Furthermore, documented evidence supports the transfer of antimicrobial traits, underscoring the extensive impact of this phenomenon across diverse boundaries and its potential to jeopardize antibiotic-based treatments in both human and veterinary medicine [28].

At first glance, it is noticeable that only *B. megaterium* was detected in both the hospital, by the maternity ward, and in the university, on dispensers by the cafeteria. In addition, the genus of *Staphylococcus* seemed to be the dominant one. Score values over 2.00, equaling high-confidence identification, were recorded in 19 out of 27 identified bacteria.

Among the identified microorganisms were species that are considered to be integral part of the skin microbiome. *S. lugdunensis*, *S. aureus*, *M. luteus*, *S. caprae*, *S. epidermidis*, *S. xylosus*, *S. warneri*, *S haemolyticus* and *S. hominis* are regular members of the diverse skin community [29–35]. However, there have been multiple cases reported where these bacterial species become major nosocomial pathogens leading to severe HAIs. *S. aureus* might be one of the most popular bacteria reported to be multi-drug-resistant and can remain on various surfaces for up to 5 years, over a wide range of temperatures, and infect patients mainly through contaminated surfaces [36–41]. In a study effort to describe the epidemiology of nosocomial outbreaks by Gastmeier et al., *S. aureus* have been found to contribute to the extent of 14.8% on 1022 nosocomial outbreaks included in the research [42]. *S. aureus* was detected on the dispenser located by the waiting room of the chemotherapy ward.

S. caprae might naturally colonize the human skin, the nails and nasal mucosa but occasionally act as an opportunistic pathogen infecting immunocompromised patients, leading to HAIs and community-acquired infections [35–43]. *S. caprae* was detected on samples taken from dispensers located by the main hospital entrance and the corridor of outpatient clinics.

M. luteus is a natural bacterial component of our human skin and besides a few reported infectious cases in immunosuppressed patient, *M. luteus* is not classified as an opportunistic or nosocomial pathogen [33,34]. *M. luteus* was detected on samples taken from dispensers of the main hospital entrance.

S. epidermidis might be a permanent resident of the human epithelial microflora, but it is also a major pathogen causing HAIs and severe infections among immunocompromised people. This bacteria has the capacity to form biofilms in surfaces that they reside in, and

subsequently to colonize patients, especially through medical devices [36,37]. *S. epidermidis* was identified on dispensers located by the main hospital entrance, the maternity ward and the corridor of outpatient clinics.

S. haemolyticus is classified as a serious nosocomial infection agent. In 2012, an outbreak of *S. haemolyticus* at the Verona University Hospital Intensive Care Unit was reported. A rather interesting study has revealed both *S. haemolyticus* and *S. hominis* species that have acquired resistant properties to all clinically important antibiotics, including those of ß-lactams [38,41]. *S. haemolyticus* and *S. hominis* were identified on dispensers located by the outpatient clinics and the maternity ward, respectively.

In summary, Coagulase-negative staphylococci (CoNS) infections, especially in hospital settings, are a significant concern due to their ability to rapidly acquire resistance to multiple antibiotics, form biofilms on medical devices and pose challenges for effective treatment. Preventative measures and surveillance are crucial in managing and controlling these infections in healthcare facilities [44,45]. CoNS are prevalent opportunistic pathogens, while also being widespread commensal microorganisms in both humans and animals. CoNS strains associated with infections in healthcare settings are often distinguished by their notable antimicrobial resistance (AMR) profiles, encompassing resistance to Methicillin and multiple other drugs [27,46] CoNS are commonly found on the skin and mucous membranes, sharing the same ecological niche in the anterior nares of humans with *S. aureus* and various other bacteria [27,44,46]. This close proximity may facilitate the transfer of resistance genes among these bacterial species [47–50]. Certainly, CoNS have been recognized as reservoirs and origins of resistance traits that are transmitted within the *Staphylococcaceae* family [27,51–53].

Furthermore, two species from the genus *Bacillus* were identified: *B. megaterium* by the cafeteria of the University and the maternity ward at the hospital and *B. cereus* by the postoperative care ward. *B. megaterium* can be found ubiquitously across diverse environments, including but not limited to soil, seawater, rivers, seafood and salt lakes. However, it is not considered a cause for nosocomial outbreaks, while up to this date mainly individual case studies were reported regarding its pathogenicity to humans [54–56].

B. cereus is ubiquitous and it can be detected in all surfaces, from soil, foods, human skin and salty water to air filtration/ventilation equipment and medical equipment. This bacterium might be associated with food poisoning, but it is also classified as a significant factor in HAIs among immunocompromised hospitalized patients [57,58]. In a cross-sectional study about bacterial cross contamination in ICUs at a French University Hospital by E. Kuczewski et al. [58], 6.3% of the bacteria isolated out of 137 samples belonged to *B. cereus* and other *Bacillus* spp. All samples were received from surfaces close to or distant to the patients [59]. The same study has also unveiled that 8.1% of the species identified pertained to *P. agglomerans* [59].

P. agglomerans environmental reservoirs include plants, water, soil, humans and animals. This bacterial species has been reported to cause HAIs of opportunistic character, mainly in hospitalized immunosuppressed hosts either from contaminated medical devices or fluids [59,60]. *P. agglomerans* was detected on dispensers by the chemotherapy ward of the hospital.

M. calida, a microorganism widely distributed in diverse environments including plants, humans and food products, was identified on dispensers by the outpatient clinics. So far, no HAI caused by *M. calida* was reported [61].

The opportunistic pathogen *P. luteola* was found and identified by the main hospital entrance dispensers. It is isolated mainly from moist environments such as soil and waters. *P. luteola* has been detected in contaminated solutions such as distilled water, disinfectants and intravenous solutions [62,63]. Severe cases attributed to *P. luteola* infections in the hospital environment have been reported and thus it is considered to be a rare but significant cause of nosocomial or community-acquired infections [64,65].

By the CAT scan clinic, *L. xylanilyticus* was identified, but reported a low-confidence score value (<2.00). This bacterium has been isolated initially from plants and no HAI has been associated with it [66,67].

S. silvestris, primarily isolated from soil and plants, was identified by the hospital's main entrance and scored a low-confidence value (<1.73), and no cases of HAIs have been reported so far. Whilst it is not considered part of the skin microflora, it has been isolated from human hands [68].

Cytobacillus oceanisediminis, detected on dispensers by the university's department of histology, and *Paenibacillus glucanolyticus*, by the university's department of hygiene, scored low-confidence identification values. They are mainly isolated from marine sediments and plants and no reference to HAIs has been reported so far [69,70].

Simon D. Eiref et al. studied the microbial load of 17 hand sanitizer dispensers by 12-bed surgical ICUs of a hospital. They detected one or more bacterial species on every dispenser and among others identified *S. saureus*, *Micrococcus* spp. and *Bacillus* spp. [71].

A limitation of this study might be that the dispensers were placed in areas which visitors, patients and hospital staff all have access to. It would be interesting if samples were taken from hand sanitizers that either only staff or only patients had access to, and to eventually compare these two findings. Also, neither the origin nor the number of dispenser users were known. Another limitation is that dispensers by the campus area are accessible to students and personnel that work both in the university and the hospital, but we do not have a record about this area. Finally, it should also be emphasized that an enrichment culture method in broth was used in the present study, which does not allow the microbial load to be determined. For this reason, we should also consider the possibility that each identified bacterial isolate may represent a single bacterial cell, so that the sampled surface may not act as a potential source of transmission. MALDI-TOF mass spectrometry analysis, on the other hand, delivers sound and valid results. It would be of great interest to define the time necessary for the alcoholic gel to eliminate all bacteria isolates identified to zero. Limitations would be addressed with the continuation of the study.

Hand hygiene is universally acknowledged as a fundamental component in infection control strategies within healthcare settings [6,7]. Physicians recognize its paramount role in preventing HAIs and safeguarding patient safety [11].

Education, along with systematic monitoring and comprehensive training protocols for all hospital personnel, is essential in promoting effective hand hygiene. These protocols must follow rigorous standards, best practices, guidelines and recommendations established by both national healthcare agencies and international health organizations such as the World Health Organization (WHO) [4–6].

The medical community is dedicated to continually improving hand hygiene practices [6,7] and ensuring the safety of hand sanitizers and dispensers [8]. Physicians actively contribute to a collective endeavor so as to enhance patient safety and reduce infection risks [6].

Manual hand sanitizer dispensers have a notable effect on hand hygiene, depending on their use and maintenance and ranging from bolstering to diminishing. Understanding their capacity for bacterial transmission is critical for practical applications. Several important factors come into play, including hand contamination and cross-contamination risk [6,7], both of which can lead to bacterial transmission. Dispensers create conditions conducive to these concerns due to the warmth of and the possibility of bacterial growth in residual products [8]. Hence, it is crucial to establish systematic maintenance and cleaning procedures firmly rooted in rigorous hygiene protocols, as well as the training of hospital staff in such practices [4,5].

Finally, it would be remiss not to mention that it is a common belief that even if the dispenser is contaminated by the hands of the user to an unknown level with skin flora, this does not pose a significant risk because the hands of the next user will be treated with the hand sanitizer after contact with the dispenser. At this point, we should digress by pointing out that factors such as heavy workload and fatigue for healthcare stuff and the

lack of proper hand hygiene training for hospitals visitors, among others factors, may lead to improper or incomplete hand disinfection, which in turn may lead to the transmission of pathogenic bacteria, a possibility that features the surfaces of the dispensers as major risk determinants.

To summarize, manual hand sanitizer dispensers serve as indispensable tools for promoting hand hygiene [6–8], yet they can unintentionally aid in bacterial transmission when not managed properly. To mitigate this risk, it is essential to underscore the importance of proper hygiene practices, regular dispenser maintenance and the investigation of alternatives that do not require direct contact.

5. Conclusions

Hospital-acquired infections (HAIs) introduce substantial risks and complexities within healthcare environments that might result in compromised patient safety and increased morbidity and mortality rates among individuals under medical care. Moreover, HAIs contribute to elevated healthcare expenses and burden hospital staff due to extended patient stays, which is not to mention that HAIs involve antibiotic-resistant bacteria, intensifying the level of concern.

In aggregate, the prevalence of HAIs can markedly influence a hospital's standing within the healthcare community. Therefore, it is essential to initiate research endeavors that delve into conceivable origins of infections within healthcare institutions and evaluate antibiotic resistance. These examinations are critical in comprehending and efficiently managing these elements with infection control strategies, ultimately diminishing HAIs, enhancing patient safety, and elevating healthcare results.

Based on the MALDI-TOF results, species of the genus Staphylococcus were the most dominant. Other identified bacteria include *Bacillus cereus*, *Bacillus megaterium*, *Pantoea agglomerans*, *Pseudomonas luteola*, *Cytobacillus oceanisediminis*, *Micrococcus luteus*, *Mixta calida*, *Lysinibacillus* spp. and *Paenibacillus glucanolyticus*. Two organisms in the hospital area could not be identified. Among the list of bacteria identified that also scored high-confidence identification scores, eight bacteria, or 29.6% of the total identified, are associated with severe nosocomial infections or outbreaks. Systematic cleaning and the correct use of manual dispenser areas are recommended to minimize the microbial load. Alternatively, automated dispensers are recommended to avoid hand touching and potential bacterial spreading. Undoubtedly, hand hygiene is an essential element for infection control strategy, and thus sanitizing gels and dispenser equipment should be scrupulously controlled for the presence of microorganisms in order to eliminate cross-contamination risks and nosocomial outbreaks. To mitigate the risk of CoNS acting as an AMR reservoir, healthcare institutions must prioritize infection control measures, including strict adherence to antibiotic stewardship programs, proper hand hygiene and the judicious use of antibiotics. Additionally, research into the molecular mechanisms of antibiotic resistance in CoNS and strategies to prevent their spread is crucial in addressing this concern and preventing the further proliferation of antibiotic-resistant bacteria.

One of the concerning aspects of CoNS is their potential to act as a reservoir for antimicrobial resistance (AMR) genes.

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