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Nosocomial Pathogens in Clinical Laboratory Departments of Various Hospitals in Ha'il, Saudi Arabia

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ABSTRACT

Healthcare-associated infections (HAI's) caused by nosocomial pathogens are accountable for high morbidity and mortality. The aim of this study was to conduct the surveillance of nosocomial pathogens in clinical laboratory departments in hospitals of Ha'il region. In this study, 255 samples were collected, of which 84% were positive for nosocomial pathogens. The total microbial isolates among the positive samples were 844 (average 3.31 bacteria per sample). The results of this study showed a high percentage of multi-drug resistant (MDR) bacteria. From the total S. aureus collected isolates, the methicillin resistant S. aureus (MRSA) was found to be 66% from the hospital environments; 55% among healthcare staff, and 42% among healthcare students. Furthermore, a high percentage of extended-spectrum beta-lactamase (ESBL) positive Escherichia coli, Klebsiella species, and Enterobacter cloacae was detected. From the environmental samples, the ESBL rate among Escherichia coli, Klebsiella species, and Enterobacter cloacae was found to be 60%, 75%, and 70% respectively. On the other hand, from healthcare-staff the ESBL rate among Escherichia coli, Klebsiella species, and Enterobacter cloacae was found to be 50%, 60%, and 50% respectively. The results of this study conclude a high rate of pathogenic organisms from various study sites (labs, HCWs, HCSs, environment, and control subjects). This gives a strong indication for the possibility of transferring other organisms, which may cause very serious problems to hospital staff as well as the community. To prevent microbial contamination, standard guidelines must be followed, and infection control strategies should be developed and implemented.

Key words: *Healthcare workers, Nosocomial pathogens, Contamination, Hand hygiene, Mobile phones, Bacteria*

INTRODUCTION

Nosocomial infections within hospitals and clinics remain an important topic of discussion amongst the healthcare community. They are commonly transmitted may be due to lack of effective infection control or lack of abiding by appropriate hospital hygiene maintenance plan regularly [1].

HAIs are accountable for high morbidity and tremendous mortality. An evaluation on the pervasiveness of HAIs directed by WHO in 2009 out of four of the six WHO regions (South East Asia, Europe, eastern Mediterranean,

and Western Pacific) discovered that 8.7% of hospitalized patients had acquired an HAI. HAIs in developed countries affect 5% to 10% of hospitalized patients. In a few developing countries, they reach 25% of hospitalized patients [1].

Despite upgrades in modern treatment options HAIs continue to be a leading and highly contagious disease problem of world health systems. The transmission of microorganisms among clinicians, patients, devices, and widespread surfaces usually defines the source. Furthermore, the hands of HCWs are often contaminated by pathogens, and insufficient hand hygiene can allow the transmission that will result in HAIs [2].

One of the most important HAIs is laboratory-acquired infections (LAIs) due to the extensive diversity of bacteria, viruses, fungi, and parasites, surveys of LAI suggest that are the most common bacterial causes *Brucella* species, *Shigella* species, *Salmonella* species, *Mycobacterium tuberculosis*, and *Neisseria meningitidis*. Diseases that are related to bloodborne microorganisms (hepatitis B infection, hepatitis C infection, and HIV infection) remain the most well-known announced viral contaminations. In 2002–2004 overview of clinical research center chiefs who take an interest in ClinMicroNet showed that 33% of laboratories detailed events of no less than one LAI. The three most normal LAIs were shigellosis, brucellosis, and salmonellosis. Interestingly, the most noteworthy frequencies of contamination were related with Brucella species (641 cases for each 100,000 lab technologists, contrasted and 0.08 cases per 100,000 people in everybody) and Neisseria meningitidis (25.3 cases per 100,000 lab technologists, contrasted and 0.62 cases per 100,000 people in everyone) [3].

Literature has defined laboratory infections owing to varied microbe (including bacteria, viruses, Rickettsiae, fungi, and parasites). The major study of infections in 1976 by Pike [4], found that 4079 LAIs were because of 159 agents. No less than 173 deaths were because of LAI [5, 6]. Nevertheless, care should be taken in the understanding of past reviews, since few infections (such as dermatomycoses, Q fever, and Venezuelan equine encephalitis) occurred mainly in research and animal laboratories, and various infections (such as typhoid and psittacosis) were reported before 1955 [3, 7].

The rapid development of modern technology has contributed not only to medicine but also to the improvement of technology for individual use. This generation consists of private computers, portable devices (Wi-Fi tablets along with iPad, androids, etc.), and mobile phones, wherein upgrades have been made at an astounding pace over the past two decades [8, 9]. However, the cell phones which we often carry in our pocket and hold with clean or dirty hands can lead to potential risks, such as noise, distractions, loss of concentration, data safety, disturbance of patient privacy, and transfer of microorganisms possibly leading to nosocomial infections [10, 11]. Microorganisms can be transmitted to mobile phones after hardly ever cleaned incorporating several resistant bacteria, after contact with the affected person, and maybe a source of bacterial cross-contamination. Furthermore, the hands of HCWs are often contaminated by pathogens, and insufficient hand hygiene can allow the transfer that will result in HAIs. A related study showed a significant result from the hand of healthcare which should be considered to limit transmission of HAI. Studies have investigated the hand carriage of aerobic Gram-negative rods [11].

Recently, infection control has been inclined to focus on patients' endogenous flora as the foremost source of HAI, with the principal route of transmission from infected and colonized patients to healthcare personnel hands. Contaminated hospital devices, drugs, and water supplies have additionally been diagnosed as other common sources of hospital outbreaks. In contrast, the dry hospital surface environment has lately been taken into consideration as a probably important source of HAIs and plays a role within the transmission of multi-drug resistant organisms (MDROs).

There is growing proof of the essential function the environment plays in in-health facility infection. Critical nosocomial pathogens can live on dry surfaces for extended durations. The predominant nosocomial pathogens, such as methicillin-resistant and -sensitive Staphylococcus aureus (MRSA, MSSA), vancomycin-resistant, and sensitive Enterococcus spp. (VRE, VSE), Clostridium difficile, Acinetobacter spp., and norovirus may survive for months on dry surfaces. Gram-negative organisms apart from Acinetobacter tend to be less resistant to drying. However, Pseudomonas aeruginosa, Klebsiella spp. can survive for lengthy intervals and this could contribute to cross-infection [12].

The fact that laboratory personnel, especially those in microbiology, are more susceptible to becoming infected than the general population has attentively focused on the factors associated with LAIs. Those factors consist of the method of transmission, the development of contamination within the host, route, source of infection, and the laboratory surroundings (e.g., airflow, equipment, and procedures) [13].

The threat of LAI in personnel of clinical and research laboratories is more than in the preferred populace, suggesting that distinctive risks are associated with the laboratory work site [13]. The purpose of this study was

to do surveillance of nosocomial pathogens in clinical laboratory departments in humans (HCW in the laboratory and clinical laboratory students) and environment to evaluate the level of contamination.

MATERIALS AND METHODS

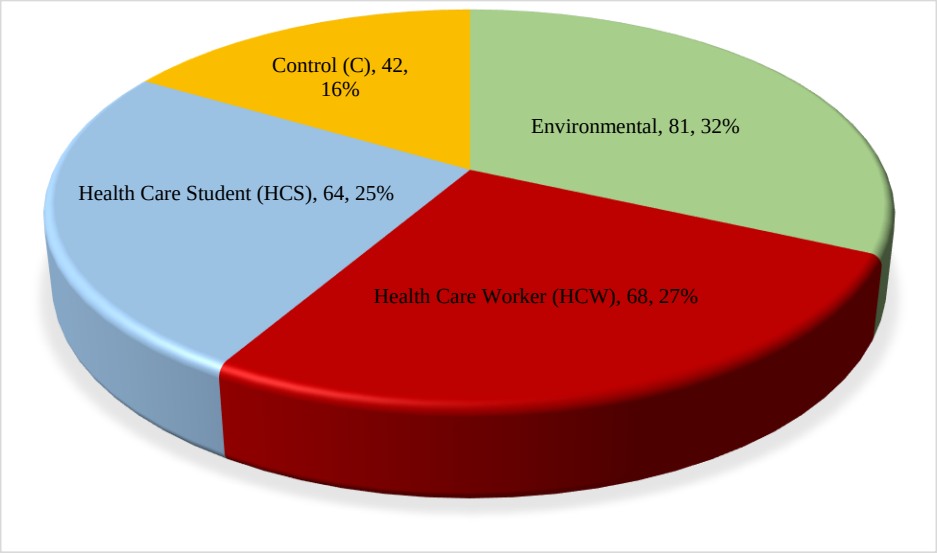
Two hundred and fifty-five (255) swabs were collected from several hospitals in Ha'il Saudi Arabia between November and December 2019. Swabs were collected from HCWs, health care students (HCSs), and control samples from volunteers who were not related to healthcare. The various samples included hands, nails, and personal belongings like cell phones, iPad, jewelers, notebooks, hand watches, ID cards, eyeglasses, lab coats, scrubs, and hijab **(Figure 1)**. Apart from this, hospital environmental samples were collected from different laboratories departments. The laboratory departments were comprised of microbiology, chemistry, phlebotomy room, staff room, offices, blood bank, and hematology **(Figure 2a)**. The samples were collected from several environmental surfaces like computer keyboards, bench, chairs, biosafety cabinet, microscope handle, centrifuge surface, autoclave handle, sink handle, diminution surface, refrigerators handle, telephone for lab office, staff room (floor, water heater, food refrigerator handle, table, and sofa), tube scales separation, glossing stage, and platelet aggregator **(Figure 2b)**. All samples were collected using sterile moisturized swabs with sterile normal saline solution.

Microbial culture and identification

Swabs were immediately inoculated in Robertson's Cooked Meat (RCM) Media and incubated at 37℃ for 24h, then cultured on blood agar, chocolate agar, MacConkey agar, and Cystine-Lactose-Electrolyte-Deficient Agar (CLED) agar. The culture plates were further incubated for another 24 h at 37℃. Screening for fungus was done by using Sabourauds plates at 30℃ for up to5 days.

Gram stains were done for all isolated bacteria; Gram-negative bacteria were sub-cultured on Chromogenic agar for the primary identity of most common pathogenic bacteria and on ESBL chromogenic agar to detect ESBL strains. Catalase test done for all Gram-positive cocci, *Staphylococcus* sp. were cultured on mannitol salt agar (MSA) to identify *S. aureus* (SA), all *S. aureus* bacteria were cultured in MRSA chromogenic agar for preliminary detection of MRSA. Bile esculin test done for all *Enterococcus* sp. and positive isolates re-cultured on VRE chromogenic agar detect Vancomycin-Resistant Enterococci (VRE).

All pathogenic bacteria were sent to specialized labs for further identification by using MALDI-TOF MS [14-18] and antibiotic resistance by using the Microscan WalkAway System [Beckman Coulter] [17, 19].



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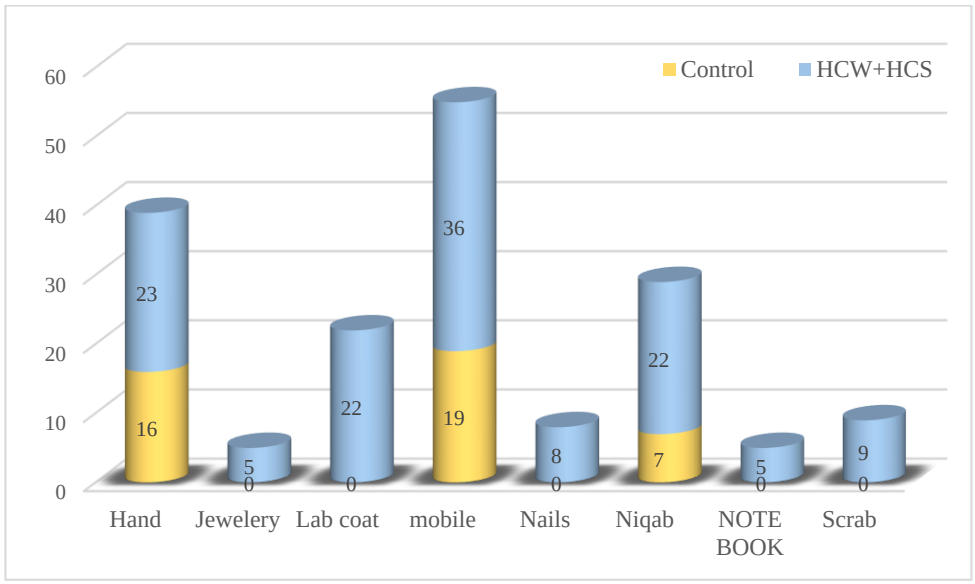

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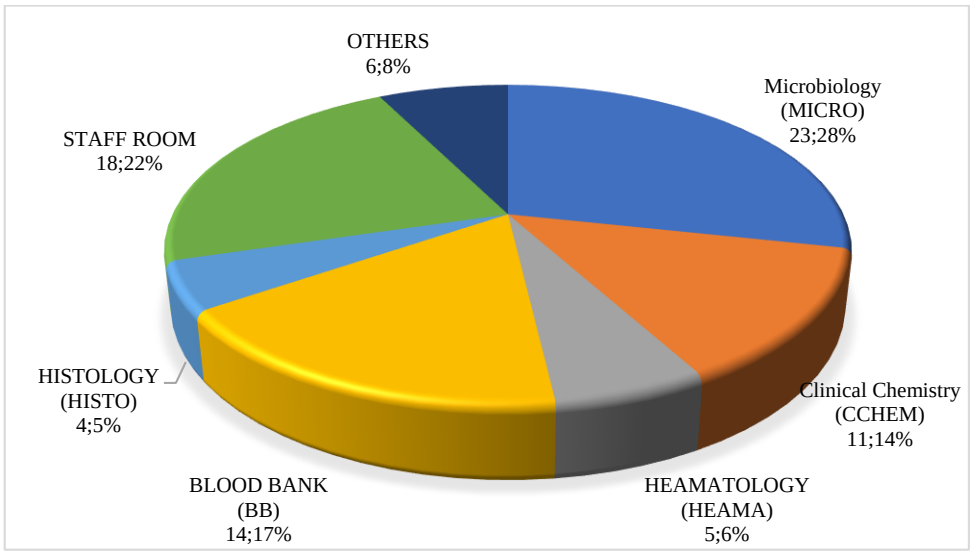
Figure 1. Distribution of samples collected from different groups (a), by screening different sites (b)



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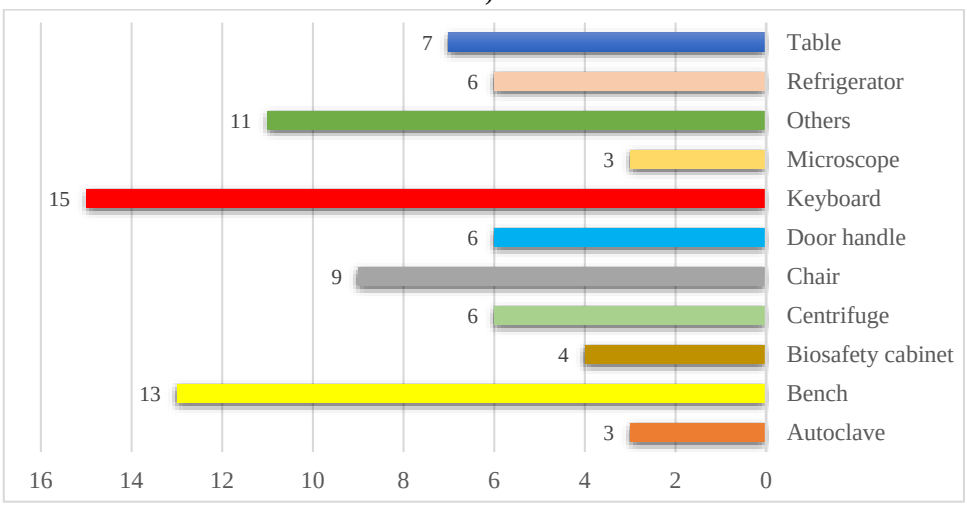

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Figure 2. Distribution of Environmental samples collected from laboratory departments (a), screening different areas in the labs (b).

RESULTS AND DISCUSSION

In this study, 84% out of 255 samples were positive for the nosocomial pathogen, and the majority of them, HCS showed the highest percent (92%) of microbial colonization. This could be attributed to lack of experience and not wearing personal protective equipment (PPE) like a mask, gowns, etc., while the control samples collected from non-healthcare related volunteers showed the lowest percentage (74%) growth because they do not have exposure to nosocomial pathogenic bacteria **(Figure 3a)**.

The 844 isolates cultured comprised of different pathogenic organisms like *S. aureus*, *E. coli*, *Klebsiella* spp., *Enterococcus faecalis*; *Candida* spp. **(Figure 3b)**. Normal flora too was isolated also and the most common bacteria were *Bacillus* spp.

S. aureus was the most common pathogenic organism isolated from all samples **(Figure 4)**. Of which 66% MRSA (highest rate) were cultured from the environmental samples and 29% MRSA (lowest rate) were from control samples **(Figure 5a)**. Similarly, the presence of ESBL in Gram-negative bacteria was documented **(Figure 5b)**.

In the staff samples, the distribution of *S. aureus* was found to be very high in all sites. It was found to be 70% in healthcare-related samples and 33% in control samples.

In niqab, the most common pathogen in healthcare-related samples was *S. aureus* (45%) followed by *Klebsiella* spp. 32%. Whereas the control samples did not reveal any *S. aureus*, instead *Klebsiella* spp. and *Enterococcus faecalis* were predominant **(Table 1)**.

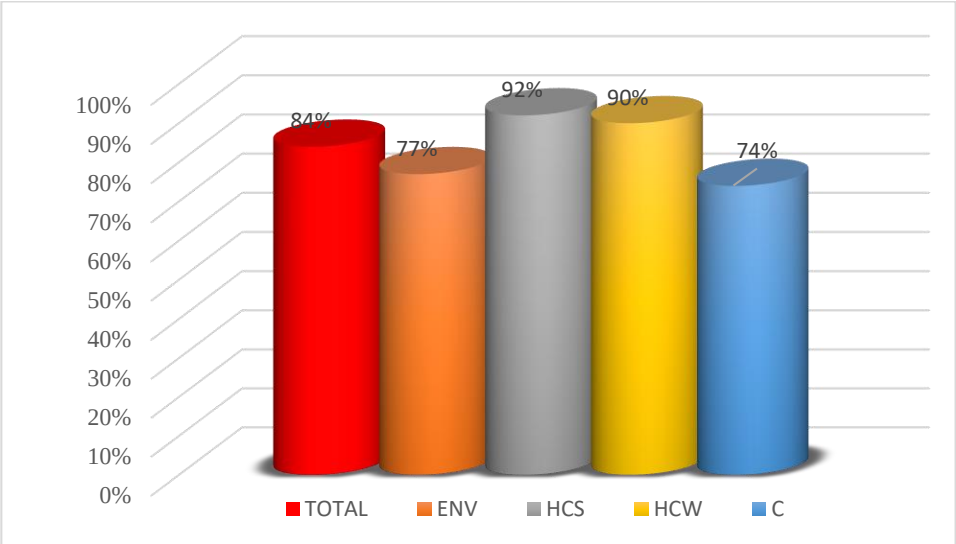
In cell phones, *S. aureus* was the common pathogenic bacteria in the hospital-related sample (77%) as well as control samples (37%).

MRSA was found in all (100%) HCSs notebooks (leather cover notebooks in the lab), which can be easily contaminated. The most important transmission source of nosocomial pathogens in the health care setting is implicated to be Hands. Swabs from staff hands showed the highest *S. aureus* rate of 83%, while in control samples the *S. aureus* and *Enterococcus faecalis* were found in the same proportion (44%).

The hospital-acquired bacteria outlined in our studies such as *Staphylococcus* spp., *Enterococcus* spp., *Pantoea* spp., *Klebsiella* spp., and *Candida* spp. have been shown to survive on inanimate surfaces even for months. Therefore, the lack of regular surface decontamination practices can be an increase in the origin of transmission in the personal items used by clinical laboratory staff and students such as cell phones, notebooks, and jewelry.

Environmental samples showed that the microbiology lab had the high rate of pathogenic organisms, which is expected because of culturing a considerably huge number of samples in the lab. On the other hand, the highest number of pathogenic microbes were cultured from the hospital staff room (66 isolates), followed by the microbiology lab (62 isolates) **(Figure 6a)**. This needs to be considered cautiously, as staff uses the hospital staff room for coffee breaks and meals. On the other hand, the lowest number of pathogenic organisms were isolated from the histology lab, with a predominance of *S. aureus* (70%) **(Figure 6b).**

The distribution of pathogenic isolates from different environmental sites ranged from 4.24% (in biosafety cabinets) to -15.9% (in keyboard), the small difference between them gives a hint that the presence of pathogens may be due to not following the proper cleaning and disinfection protocols (**Figure 6b**).



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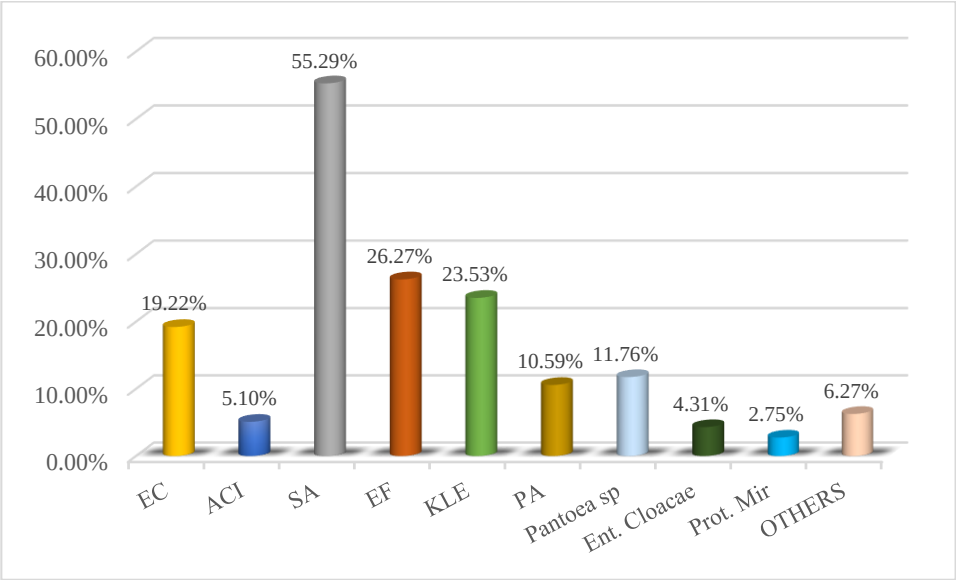


b)

Figure 3. Distribution of nosocomial pathogens among the different human samples (a), and distribution of isolated organisms (b). EC- *Escherichia coli*; ACI- Acinetobacter spp.; SA- *Staphylococcus aureus*; EF- *Enterococcus faecalis*; KLE- *Klebsiella* species; PA- *Pseudomonas aeruginosa*; Ent. Cloacae - *Enterobacter cloacae*; Prot. Mir- *Proteus mirabilis*.

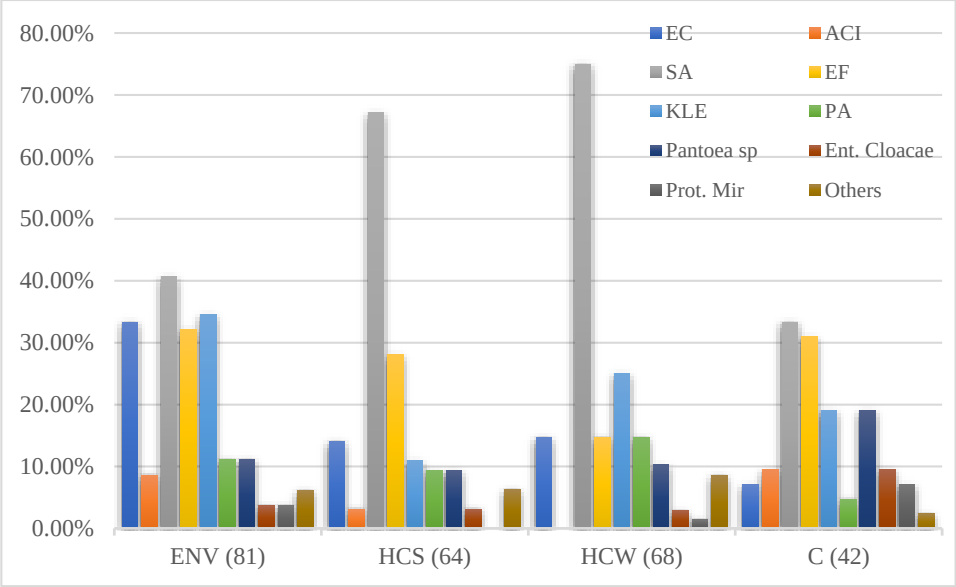
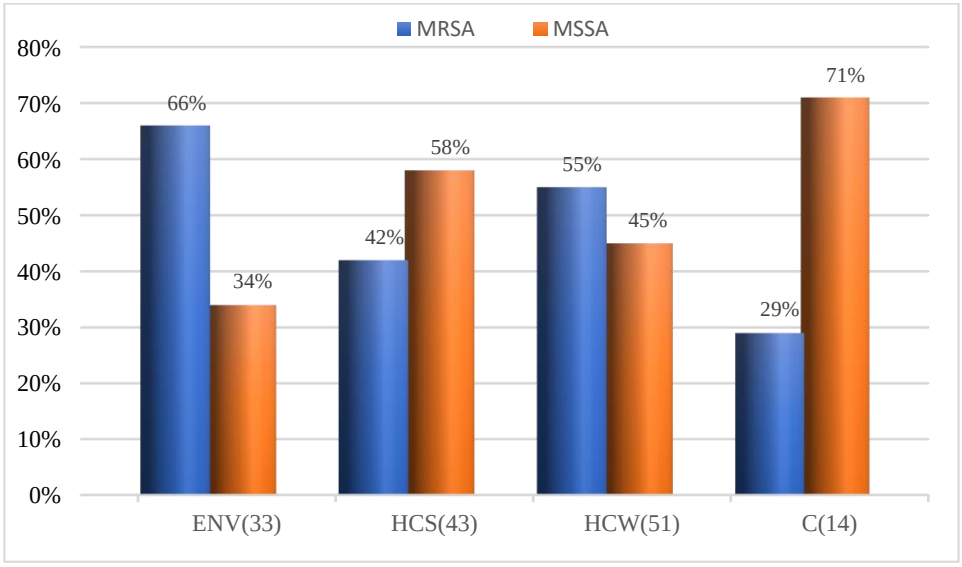


Figure 4. Pathogenic organisms are isolated according to the type of sample.
EC- *Escherichia coli*; ACI- Acinetobacter spp.; SA- *Staphylococcus aureus*; EF- *Enterococcus faecalis*; KLE- *Klebsiella* species; PA- *Pseudomonas aeruginosa*; Ent. Cloacae- *Enterobacter cloacae*; Prot. Mir- *Proteus mirabilis*.



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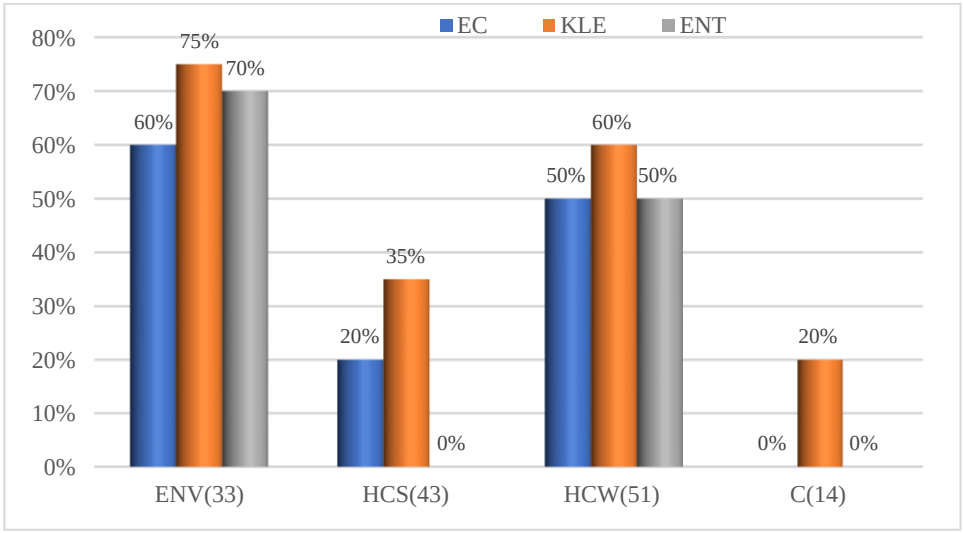


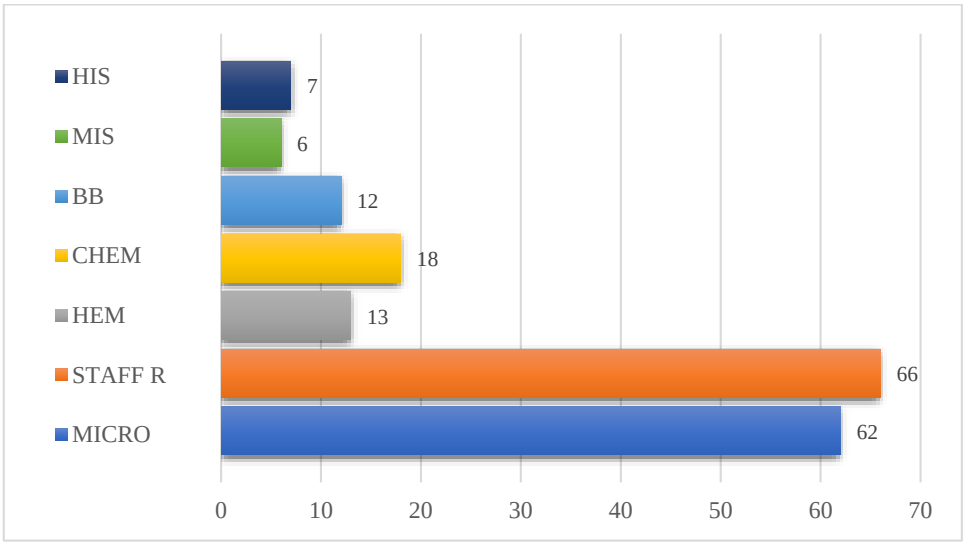
b)

Figure 5. Multidrug-Resistant Bacteria according to the type of sample, Methicillin-resistant *Staphylococcus aureus* (a) and Beta-lactamase extended-spectrum (b). EC- *E.coli*; ACI- Acinetobacter spp.; SA- *Staphylococcus aureus*; EF- *Enterococcus faecalis*; KLE- *Klebsiella* species; PA- *Pseudomonas aeruginosa*; Ent. Cloacae - *Enterobacter cloacae*; Prot. Mir- *Proteus mirabilis*; Methicillin-resistant *Staphylococcus aureus* (MRSA)*;* Methicillin-sensitive *Staphylococcus aureus* (MSSA).

Table 1. Distribution of Isolated nosocomial pathogenic from health care workers (HCW) and control samples from non-Health care related staff (NON-HCW).

	HCW									NON-HCW			
Samples / **Positive (%)**	**Hand 23**	**Jewelers 5**	**Lab coat 22**	**Cell phones 36**	**Nails 8**	**Niqab 22**	**Notebook 5**	**Scrub 9**	**Total 130**	**Hand 16**	**Niqab 7**	**Cell phones 19**	**Total 42**
EC	2(9)	1(20)	5(23)	4(11.1)	0(0)	4(18)	1(20)	3(33)	20(15)	0(0)	3(43)	0(0)	3(7)
ACI	0(0)	0(0)	0(0)	0(0)	1(13)	0(0)	1(20)	0(0)	2(2)	2(13)	0(0)	2(11)	4(10)
SA	19(83)	3(60)	13(59)	28(77.8)	5(63)	10(45)	5(100)	8(89)	91(70)	7(44)	0(0)	7(37)	14(33)
EF	2(9)	2(40)	2(9)	12(33.3)	4(50)	2(9)	0(0)	3(33)	27(21)	7(44)	4(57)	2(11)	13(31)
KLE	2(9)	1(20)	6(27)	6(16.7)	1(13)	7(32)	1(20)	2(22)	26(20)	0(0)	4(57)	4(21)	8(19)

PA	1(4)	1(20)	4(18)	4(11.1)	0(0)	4(18)	0(0)	2(22)	16(12)	0(0)	2(29)	0(0)	2(5)
***Shigella* spp.**	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
***Pantoea* spp.**	1(4)	0(0)	6(27)	3(8.3)	0(0)	2(9)	0(0)	2(22)	14(11)	1(6)	3(43)	4(21)	8(19)
Ent. Cloacae	0(0)	0(0)	0(0)	1(2.8)	0(0)	1(5)	0(0)	1(11)	3(2)	2(13)	1(14)	1(5)	4(10)
Kluyvera intermedia	1(4)	0(0)	0(0)	1(2.8)	0(0)	0(0)	0(0)	1(11)	3(2)	0(0)	0(0)	0(0)	0(0)
Serratia spp.	1(4)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	1(11)	2(2)	0(0)	1(14)	0(0)	1(2)
Escherichia vulneris	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Pseudomonas luteola	0(0)	0(0)	1(5)	0(0)	0(0)	2(9)	0(0)	0(0)	3(2)	0(0)	0(0)	0(0)	0(0)
CAN	0(0)	0(0)	1(5)	0(0)	0(0)	0(0)	1(20)	0(0)	2(2)	0(0)	0(0)	0(0)	0(0)
Proteus Mirabilis	0(0)	0(0)	0(0)	0(0)	0(0)	1(5)	0(0)	0(0)	1(1)	0(0)	0(0)	3(16)	3(7)



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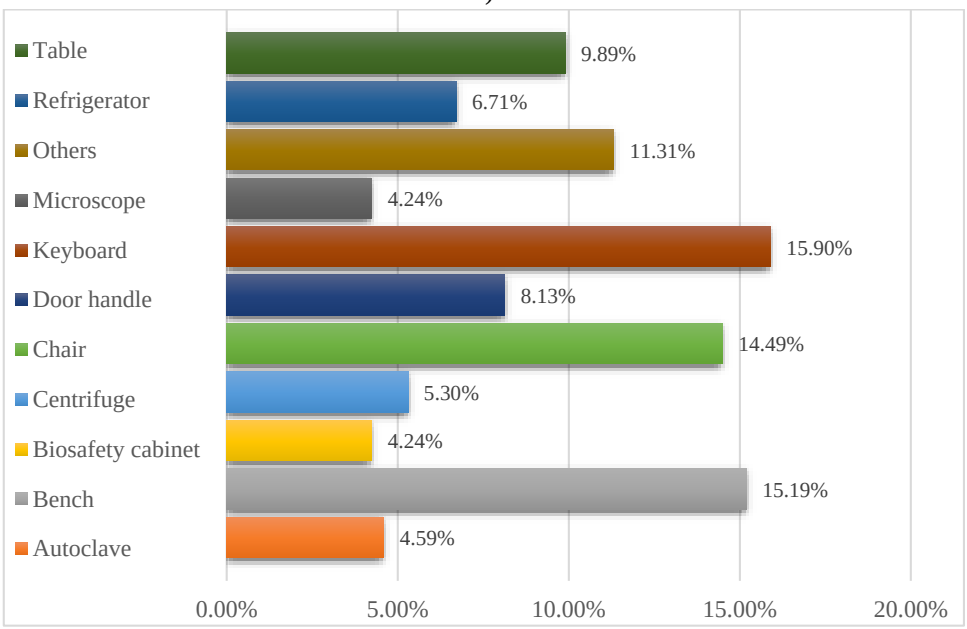

b)

Figure 6. Distribution of isolated pathogenic organisms from environmental samples (a) and different environmental sites (b).

CONCLUSION

Our preliminary results specify the presence of a high rate of pathogenic organisms from various study sites (labs, HCWs, HCSs, environment, and control subjects). This gives a strong indication for the possibility of transferring other organisms, which may cause very serious problems to hospital staff as well as the community.

Considering the COVID-19 pandemic, there is the threat of infection being spread through samples to be processed in the laboratories. Elevation of clinical activity has been proven to increase total bacterial counts on the hands of medical personnel, consequently preserving appropriate hand hygiene is vital to decrease nosocomial pathogens. During outbreaks especially among health care workers, Simple handwashing is effective in decreasing the transmission of pathogenic bacteria and viruses [8].

Nosocomial pathogens incorporating bacteria, viruses can endure on inanimate surfaces for lengthy durations. Hand washing is understood to curb disease transmission in both healthcare and community settings. The transfer of microorganisms between clinicians, patients, devices, and general surfaces usually defines the source. HCWs routinely and often contaminate their hands with pathogens, and insufficient hand hygiene can allow the transmission that will result in HAIs. The World Health Organization (WHO) has recommended regular handwashing with either an alcohol-based hand rub or with soap and water, the number of people infected with coronavirus growing around the globe daily. The hope is that good hand hygiene will limit the spread of the virus and other pathogens.

Microorganisms may spread through after rarely handling cleaned Cell phones, incorporating numerous resistant bacteria, after contact with the patient, and can be an origin of bacterial cross-contamination, regulations around the utilization and disinfection of cell phones needs to be developed. Wearing unnecessary personnel items like watches, jewelry, rings, etc. will accumulate the pathogenic organisms and it will be difficult to disinfect them and need to develop regulations around wearing them.

The environment should have regular cleaning and documentation needed to make sure it is done in the right way. Finally doing proper hand hygiene, wearing the necessary PPE, carrying out continuous training, and auditing laboratory safety will make the lab safer.

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ETHICS STATEMENT : None

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