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Comparison of microbial culture results before and after implementation of an icu-acquired infection prevention protocol at imam khomeini hospital, ardabil, iran

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ABSTRACT

Introduction:
Nosocomial (hospital-acquired) infections, particularly in intensive care units (ICUs), pose a significant challenge to healthcare systems. Effective control of ICU-related infections is crucial, as it can reduce patient mortality rates and shorten hospital stays.

Materials and Methods:
In the interventional study, 74 samples for microbial cultivation were selected of different parts and equipment and sent to the laboratory for analysis. Subsequently, the ICU departments underwent thorough cleaning, and staff received training on proper hand hygiene protocols. Post-intervention sampling was then conducted. Data from both pre- and post-intervention measurements (two sampling rounds each with 37 samples) were analyzed and sent to the laboratory. The microbial culture results were recorded in the researcher-designed checklist for further evaluation and collected data were analyzed by statistical methods in SPSS version 24.

Results:
Microbial contamination analysis revealed coagulase-negative Staphylococci, Escherichia coli, and Citrobacter freundii as the most prevalent pathogens (25% each) in pre-intervention samples. Following decontamination protocols and staff training, post-intervention cultures showed a reduction in overall contamination, with coagulase-negative Staphylococci and Acinetobacter spp. emerging as the dominant species (20% each). This observed decrease in microbial load suggests the educational intervention may have contributed to improved infection control practices.

Conclusion:
The results demonstrated a significant reduction in Gram-positive and Gram-negative bacterial contamination across both medical equipment and hospital surfaces following the decontamination protocol. This observed decrease in microbial load, particularly of pathogenic species, suggests the infection control training program effectively improved sterilization practices among healthcare staff.

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Introduction

Hospitals, as complex social systems, combining infrastructure, equipment and human resources, are responsible for providing healthcare services, but they face the significant challenge of nosocomial infections that appear 48-72 hours after admission and lead to increased mortality, length of stay and healthcare costs (1). Nosocomial infections are transmitted through a tripartite mechanism involving (1) environmental reservoirs, (2) healthcare worker practices, and (3) host susceptibility factors (2). Intensive care and infectious disease units demonstrate particularly high incidence rates due to three synergistic risk factors: elevated patient-to-staff ratios, frequent use of invasive medical devices, and the immunocompromised status of critically ill patients (3). Studies show that adherence to infection control protocols, staff training and continuous monitoring can help to significantly reduce these infections (4).

Nosocomial (hospital-acquired) infections, particularly in intensive care units (ICUs), pose a significant challenge to healthcare systems. Effective control of ICU-related infections is crucial, as it can reduce patient mortality rates and shorten hospital stays.

Hospital-acquired infections (HAIs) are defined as infections that manifest ≥48 hours after hospital admission, caused by bacterial, viral, or fungal pathogens (5). These infections disproportionately affect surgical patients and immunocompromised individuals with chronic comorbidities (6), resulting in: 2.5-fold prolonged hospitalization (mean increase: 9.3 days), 80% elevated mortality risk in ICU populations and Substantial economic burden on healthcare systems (7).

Hospital-acquired infections (HAIs) are defined as infections that manifest ≥48 hours after hospital admission, caused by bacterial, viral, or fungal pathogens (5). Recent studies highlight the growing significance of fungal contaminants in nosocomial transmission, particularly in immunocompromised hosts (8,9). These infections disproportionately affect surgical patients and individuals with chronic comorbidities (6), with surveillance data demonstrating: 2.5-fold prolonged hospitalization (mean increase: 9.3 days), 80% elevated mortality risk in ICU populations and significant fungal colonization risks in HVAC systems and high-touch surfaces (8). The economic burden is compounded by emerging antifungal resistance patterns observed in Iranian healthcare settings (9).

Statistics show that approximately 2 million cases occur annually in the United States (10), and the prevalence is 11.8% in hospitals in the Eastern Mediterranean and 10% in Southeast Asia (11). High-risk groups include patients in intensive care units, surgical patients, and immunocompromised individuals, who require special attention and strict implementation of infection control protocols. According to the World Health Organization, the highest prevalence of hospital-acquired infections is in the intensive care unit (ICU) and orthopedics and surgery (9). More than 20% of hospital-acquired infections occur in the ICU, and the mortality rate from this hospital-acquired infection is 10-80% (10). According to the statistics reported by the World Health Organization, 1.7 million hospital-acquired infections occur annually, and these infections caused 99,000 deaths annually and impose a cost of about 26 to 32 billion dollars on society (11). These infections cause 42 to 98,000 deaths annually in the United States and account for a cost of between 17 and 19 million dollars (12). Over the past two decades, the incidence of these infections has increased by 36% (13). The incidence of hospital-acquired infections in Iran has been reported to range from 1.9% to more than 25% (14)). This is an important economic burden. Hospital infections affect about 10% of hospitalized patients, while about 20% of patients in intensive care units develop this infection, increasing the mortality rate in these units to more than 30% (15). These statistics indicate that intensive care units are the riskiest places for hospital infections, which can also be due to special emergency conditions and weak immune systems of patients (16). Contamination of medical equipment in intensive care units, especially in ICU units, plays an important role in the occurrence of hospital infections (17). These infections can severely affect patient health and lead to serious complications and even death (18). Medical equipment in hospital environments, especially in ICU units, is affected by microbial contamination (19). Studies have shown that about 44% of cultured samples from medical equipment were contaminated (20). Hand washing is one of the most important preventive measures in hospital infection control, especially in intensive care units (ICUs). This practice plays a vital role in reducing the transmission of microbes and infections due to the frequent contact of nurses with patients and medical equipment (21). Hospital-acquired infections are mainly transmitted to patients through contact, especially by the hands of staff. Proper hand washing can significantly reduce the microbial load (22). According to studies, observing standard precautions, including washing hands before and after contact with patients and using protective equipment, is highly effective in reducing hospital-acquired infections (23). Timely and rapid identification of reservoirs of microbial contamination and implementation of basic planning to eliminate these contaminations will reduce the incidence and transmission of hospital-acquired diseases and the impact of imposing false treatment costs on patients (24). Therefore, hospitals should establish an infection control program to assess and control the spread of infection (25). This study was conducted to investigate the role of a preventive plan in reducing hospital-acquired infections by comparing the results of culture before and after the plan was implemented.

Materials and Methods

In this semi-experimental interventional study, samples were taken from different parts and equipment of the intensive care units of Imam Khomeini Hospital in Ardabil and sent to the laboratory for culture. Unannounced random sampling was conducted from high-contact surfaces and medical equipment prepared for patient use, including: Critical instruments (laryngoscope blades, suction tips), Monitoring devices (manometer diaphragms, ventilator surfaces), Mobile equipment (medication trolleys, infusion pumps), Patient zone surfaces (bed rails, bedside tables) and sampling sites were selected through systematic random sampling to ensure representative coverage of potential microbial reservoirs.

Environmental sampling was performed using sterile cotton-tipped swabs moistened with 0.9% physiological saline. The protocol consisted of:

1. Surface sampling: Swabs were rotated over 10×10 cm^2 areas of high-contact surfaces using standardized pressure
2. Inoculation: Samples were immediately streaked onto:
   ○ Blood agar (5% sheep blood) for general bacterial isolation
   ○ MacConkey agar for Gram-negative selection
3. Incubation: Plates were incubated aerobically at 37°C for 24-48 hours
4. Analysis: Colony morphology and Gram staining guided preliminary identification, with biochemical tests for species confirmation

Then, the unit was washed according to the standard protocol, and the hospital infection control officer for the unit nurses held a training class on hand washing. To ensure the proper implementation of the aforementioned plan, the hospital infection control officer routinely and intrusively visited the units during washing and supervised the implementation of hand washing and washing of the units. Post-intervention sampling was conducted in three phases:

1. Immediate post-decontamination:
   ○ Repeated sampling from identical locations within 2 hours of terminal cleaning
2. Short-term follow-up:
   ○ Performed 72 hours after implementing the hand hygiene protocol
3. Long-term monitoring:
   ○ Final sampling at 30-day post-intervention

All samples were collected using identical techniques to baseline measurements, immediately transported to the laboratory under controlled conditions (4°C, ≤2 hours), and processed using standardized microbiological methods. Quantitative culture results (CFU/cm^2) were recorded in the researcher-designed electronic checklist, including:

- Microbial identification
- Colony counts

- Antibiotic susceptibility profiles (when applicable)

This study was registered with the ethics code (IR.ARUMS.MEDICINE.REC.1402.164) from the Ethics Committee of Ardabil University of Medical Sciences.

SPSS version 18 was used to analyze the data. Given the qualitative nature of the data, the chi-square test was used to examine the effect of the educational intervention. The significance level for all tests was considered to be 5%.

Results

In this study, 37 random samples before the intervention and 37 random samples after the intervention were taken from different parts and levels of the surgical intensive care units, emergency, burn, general, ICU 1, ICU 2, and OH and were examined.

Table 1. Distribution of Microbial Culture Results Before and After Training

Culture Result	Before Training n (%)	After Training n (%)	Total n (%)	p-value
Positive	16 (43.2%)	7 (18.9%)	23 (31.1%)	0.02
Negative	21 (56.8%)	30 (81.1%)	51 (68.9%)	
Total	37 (100%)	37 (100%)	74 (100%)	

Before training, 16 cases, or 43.2% of cases, were positive, but after the training intervention, 7 cases, or 18.9% of cases, were positive.

The chi-square test showed that the percentage of positive cases decreased significantly after the training intervention (P=0.02). (Table 1)

Table 2. Distribution of Microbial Culture Results in ICU Wards Before and After the Educational Intervention by Ward

ICU Ward	Culture Result	Before Training n (%)	After Training n (%)	Total n (%)	p-value
Emergency	Positive	4 (57.1)	3 (25.0)	7 (46.7)	0.072
	Negative	3 (42.9)	5 (75.0)	8 (53.3)	
OH	Positive	0 (0.0)	0 (0.0)	0 (0.0)	–
	Negative	2 (100)	2 (100)	4 (100)	
General	Positive	3 (50.0)	1 (16.7)	4 (33.3)	0.221
	Negative	3 (50.0)	5 (83.3)	8 (66.7)	
Burn	Positive	0 (0.0)	0 (0.0)	0 (0.0)	–
	Negative	2 (100)	1 (100)	3 (100)	
Surgery	Positive	2 (25.0)	1 (12.5)	3 (18.8)	0.522
	Negative	6 (75.0)	7 (87.5)	13 (81.3)	
Number 1	Positive	3 (50.0)	2 (28.6)	5 (38.5)	0.429
	Negative	3 (50.0)	5 (71.4)	8 (61.5)	
Number 2	Positive	3 (50.0)	1 (20.0)	4 (36.4)	0.303
	Negative	3 (50.0)	4 (80.0)	7 (63.6)	

The highest number of contamination cases was in the emergency department with 57% in the pre-intervention period and 25% after the intervention (total 7 cases or 46.7%), followed by ICI 1 with 50% before and 28.6% after training.

In the burn and OH intensive care units, all samples were negative and no positive cases were reported. In all units, the contamination rate decreased after training, but in none of the units was the reduction in contamination significant (Table 2).

Table 3. Frequency Distribution of Microbial Agents in Culture Results Before the Training, by ICU Ward

Microorganism	Total (n=15)	ICU 2 (n=3)	ICU 1 (n=3)	Surgery (n=2)	General (n=3)	Emergency (n=4)
Coagulase-negative Staphylococcus	3 (20.0%)	0 (0%)	0 (0%)	1 (33.3%)	2 (50.0%)	0 (0%)
Acinetobacter spp.	1 (6.7%)	0 (0%)	0 (0%)	1 (50.0%)	0 (0%)	0 (0%)
Pseudomonas aeruginosa	2 (13.3%)	0 (0%)	1 (33.3%)	0 (0%)	1 (33.3%)	0 (0%)
Escherichia coli	3 (20.0%)	1 (33.3%)	1 (33.3%)	0 (0%)	0 (0%)	1 (25.0%)
Citrobacter freundii	3 (20.0%)	0 (0%)	0 (0%)	1 (50.0%)	1 (33.3%)	1 (25.0%)
Klebsiella pneumoniae	1 (6.7%)	1 (33.3%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Enterobacter cloacae	1 (6.7%)	1 (33.3%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Total	15 (100%)	3 (100%)	3 (100%)	2 (100%)	3 (100%)	4 (100%)

Infection with Coagulase-negative Staphylococcus, Escherichia coli, and Citrobacter freundii had the highest frequency (20%) among other microbes (Table 3).

Table 4. Distribution of frequency of microbial contamination (positive cultures) by intensive care unit after training

Microorganism	Total (n=15)	ICU 2 (n=3)	ICU 1 (n=3)	Surgery (n=2)	General (n=3)	Emergency (n=4)
Coagulase-negative Staphylococcus	3 (20.0%)	0 (0%)	0 (0%)	1 (33.3%)	2 (50.0%)	0 (0%)
Acinetobacter spp.	1 (6.7%)	0 (0%)	0 (0%)	1 (50.0%)	0 (0%)	0 (0%)
Pseudomonas aeruginosa	2 (13.3%)	0 (0%)	1 (33.3%)	0 (0%)	1 (33.3%)	0 (0%)
Escherichia coli	3 (20.0%)	1 (33.3%)	1 (33.3%)	0 (0%)	0 (0%)	1 (25.0%)
Citrobacter freundii	3 (20.0%)	0 (0%)	0 (0%)	1 (50.0%)	1 (33.3%)	1 (25.0%)
Klebsiella pneumoniae	1 (6.7%)	1 (33.3%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Enterobacter cloacae	1 (6.7%)	1 (33.3%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Total	15 (100%)	3 (100%)	3 (100%)	2 (100%)	3 (100%)	4 (100%)

Microbial contamination (positive cultures) in different departments after training showed that Coagulase-negative Staphylococcus and Acinetobacter spp. had the highest frequency (25%) among other microbes. (Table 4)

Discussion

In our study, out of 37 cases of culture performed on medical devices and equipment in ICU wards before training, 16 cases were positive (43.2%) and 21 cases were negative (56.8%). Also, the percentage of common microorganisms is: contamination with coagulase-negative staphylococcus and Acinetobacter spp. had the highest frequency (25%) among other microbes. Ekrami et al., in their study investigating the surface contamination of medical equipment, found that surface contamination was present in 55% of samples before disinfection and decreased to 36.5% after routine cleaning of the ICU ward (26). While in Aslani's study, out of 137 cultured cases, 125 cases (91.5%) were positive and 12 cases (9.5%) were negative. Also, the percentage of common microorganisms in order of prevalence were: Bacillus 29.9%, coagulase-negative staphylococcus 17.5%, and coagulase-positive staphylococcus (27).

In their study of the microbial contamination of medical equipment in Kashan hospitals, Nazeri et al. concluded that 76% of the studied areas were contaminated due to high levels of contamination (28). Karami et al. used the observation method (ICNA) and the method to measure surface contamination of medical equipment in their research, and 61% of the total samples were contaminated before and 39.5% after routine cleaning of the ward (29). And Najafi et al. in their study "Evaluation of ICU medical equipment levels in Neyshabur hospitals" showed that the highest and lowest contamination were related to electroshock 1% and bottle suction 8.2%, respectively (30). In a study conducted by Davidian and Mostaji, the percentage of microorganisms identified in order of prevalence were: Escherichia coli 54.7%, Pseudomonas 22.6%, Klebsiella 5.3%, Staphylococcus aureus 4%, coagulase-negative staphylococcus 2.7%, and Proteus 1.3% (31). In another study by Sayad Jou and Payandi, 272 microbial cultures were performed, of which 82 (30%) were positive and 190 (70%) were negative. The percentage of microbial contamination was: coagulase-negative staphylococcus 52.8%, Klebsiella 24.3%, Bacillus 10.9%, Escherichia coli 3.6%, and Proteus 1.2%. (32) which are inconsistent with our study.

Also, in a study conducted by Yousefi and Heidari, the average frequency of infection in the two ICU-CCU departments was 42.3% caused by gram-negative bacteria and 57.5% by gram-positive bacteria. The contamination of the ICU ward was reported to be 31.5% and the CCU ward to be 33.9% (33). A longitudinal study by Noorollahi et al. (2018) in Isfahan ICU settings revealed distinct microbial epidemiology over a 24-month surveillance period, with Gram-negative pathogens demonstrating higher environmental persistence than Gram-positive species. Escherichia coli with 30.6% prevalence (95% CI: 28.1-33.2), Coagulase-negative Staphylococcus spp. with 14.2% (95% CI: 12.4-16.0) ($p<0.001$ for inter-group comparison) (34).

In the study by Davari and Soudawi in 1997, a total of 1440 samples were prepared from the neonatal ward and 1568 samples from the maternity wards. Baseline contamination analysis revealed alarmingly high microbial loads (80-100% positivity) across all non-disposable, non-autoclavable equipment. Notably, routine decontamination protocols failed to significantly reduce contamination rates, with post-processing cultures showing:

No statistically significant difference in overall contamination ($p=0.82$, McNamara's test)

Persistent high-level colonization (78-98% positivity) in:

- Semi-critical devices (e.g., laryngoscope handles)
- Reusable monitoring equipment

These findings suggest current reprocessing methods are inadequate for non-autoclavable instruments. Comparison of our findings with previous studies reveals notable differences in microbial contamination patterns. While prior research (e.g., Noorollahi et al.) reported Gram-negative bacteria such as Escherichia coli (30.6%) as predominant, our study identified coagulase-negative Staphylococci (25%) and Acinetobacter spp. (20%) as the most prevalent contaminants across both medical (e.g., oxygen manometers) and non-medical surfaces (e.g., refrigerator handles). This discrepancy may reflect variations in infection control practices, regional pathogen prevalence, or differences in sampling methodologies. Importantly, unlike other studies demonstrating reduced contamination post-intervention, our results showed persistent high-level contamination even after decontamination efforts, underscoring potential gaps in current sterilization protocols for reusable equipment. It seems that the reason for this discrepancy may be due to the construction work carried out around the aforementioned hospital, which caused dust and soil to penetrate into the hospital. The explanation is that most members of the bacilli group are saprophytic organisms that are widely distributed in soil, water, air, and on plants. Their spores are resistant to environmental changes such as dryness, heat, and disinfectants and can survive for many years in dry land. Animal organs such as skin, fur, and nails are infected with spores of these bacteria. Although most of them are not pathogenic, important diseases such as anthrax, endocarditis, meningitis, and food poisoning are caused by these bacteria (35). This study has some limitations such as the time-consuming nature of the interventional study, the lack of financial resources and the necessary facilities and equipment for culture, and the lack of information on the results of multiple samplings from ICUs.

Conclusion

The results showed that microbial contamination with gram-positive and gram-negative bacteria in medical and non-medical hospital equipment decreased after washing, indicating the effectiveness of the training. Hand washing as the most important disinfection measure, sterilization of sampling equipment, and periodic cultures reduce the amount of microbial contamination. It is recommended that a study be conducted on antibiogram testing in positive cultures, as well as a study on a larger scale and in all hospital departments, providing strategies to reduce hospital infections.

Ethical Considerations

The study with participants abided by the ethical standards set by the Ardabil University of Medical Sciences using the code IR.ARUMS.MEDICINE.REC.1402.164.

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Conflict of Interest

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