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Research Article

Comparative Assessment of Detecting Bacterial Populations on the Surface of Medical Equipment in ICU by Standard Microbial Culture and Nanosensor

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Abstract

Background: A healthy, clean, and secure environment is necessary for the hospital, one of the fundamental foundations of the nation's healthcare system, to function well and sustain the general well-being of society. Timely detection of contaminated surfaces and efficient and timely disinfection will be helpful in hospital infection control.

Objectives: The level of surface contamination of medical equipment in intensive care units was to be determined and compared as part of this research.

Methods: Using standard microbial culture and nanosensors, the current study was conducted descriptively over one month, with a sample size of 400 cases on ten different types of medical equipment.

Results: The findings showed that 66% of samples acquired using the nanosensor and 54.5% obtained using the culture medium were clean, and the rest were contaminated. The most prevalent microbes were also identified as *E. coli, Staphylococcus aureus,* and salmonella, with 55.68%, 28.9%, and 23.86%, respectively.

Conclusions: Both methods have the necessary precision to identify contamination reservoirs, and the contamination reported in both methods is similar to what was expected. So nanosensors can be utilized as a quick, precise, and affordable method when the aim is to identify the overall contamination rather than to differentiate between different types of bacteria.

Keywords: Hospital Infection; Healthcare-Associated Infections; Nanotechnology; Infection Control; Indicators; Reagents

1. Background

One of the most intricate social systems is the hospital, which uses infrastructure, equipment, and human resources to offer public diagnosis, treatment, and rehabilitation services. Patients expect hospitals to provide safe, excellent, and efficient services since the World Health Organization describes hospitals as locations where health is prioritized over disease (1-3). Despite the development of new medical techniques, particularly in the field of performing complex surgeries, the issues of environmental health and infection in the centers have affected the outcomes of therapeutic interventions. That's why the late 16th-century topic of environmental health and control of hospital infections has received the attention of thinkers and policymakers in this field (4-8). Healthcare-associated infections (HCAIs) healthcare-acquired infections that develop in a hospital or other healthcare setting first occur 48 hours or more after hospital admission or within 30 days. They appear after receiving healthcare (9). The prevalence of nosocomial infections has emerged as one of the major issues facing the nation's healthcare system today. Nosocomial infections are crucial for infection, attenuation, and expense (10-12). These infections prolong the length of hospital stays, dramatically increasing healthcare costs and eventually resulting in patient discontent, death, and consequences. Each year, these illnesses place a substantial financial strain on the healthcare system, as well as on patients and those who treat them (13-15). According to the US Centers for Disease Control and Prevention, nearly 1.7 million hospitalized patients receive HCAIs yearly while being treated for other health problems (16, 17). The timely and quick diagnosis of microbial contamination reservoirs and implementation of fundamental planning to eliminate these contaminations will lower the occurrence and transmission of hospital illnesses and the impact of imposing false treatment expenses on patients (7, 18). Therefore, hospitals should develop an infection control program to evaluate and control the spread of infection (19, 20). Among the high-touch environmental surfaces (HTES), the surfaces of medical devices and equipment inside the hospital are among the most important and direct factors in the transmission of microorganisms that cause HCAIs to hospitalized patients. Periodically, in the shortest time and with the most accurate methods, the level of surface contamination should be monitored so that if the level of contamination exceeds the standard level, necessary warnings will be given, and a fundamental review of the internal surface cleaning programs (3, 21). Currently, the control of pathogens causing hospital infections is routinely carried out by the sub-units of the Infection Control and Quality Improvement Unit with traditional methods, including standard microbial culture medium. Traditional methods based on microbial culture have high sensitivity and reproducibility. However, these techniques require laborious processes of sample preparation and long rereading and time delay to detect contaminations, specialized force, high cost, and relatively long time, and therefore, it will be a challenge in cases where rapid diagnosis is necessary (22-24), so the necessity the use of simple, fast and up-to-date technologies, such as nanotechnology, is felt more to detect pollution. Recently, scientists have made significant progress in manufacturing nanofibers and nanocomposites with different capabilities and have been developing and manufacturing fast, cheap, and accurate nano-detector sensors (23, 25-36).

2. Objectives

This study was done as a comparative assessment of detecting bacterial populations on the surface of medical equipment in intensive care units (ICU) by standard microbial culture and nanosensor.

3. Methods

The ICU at Tehran's Imam Khomeini Hospital complex were the site of this descriptive study, which was conducted there for a month, randomly twice a week. P = 0.025, D = 0.043, and 95% confidence level were used to calculate the sample volume, which was generated using the volume calculation formula (Cochran formula) and 400 samples were generated using each method. First, the researcher chose ten different types of medical devices with a high probability of contamination based on previous studies (23, 37, 38); then, sampling was done simultaneously with each method.

3.1. Monitoring by Nano Sensors

The electrospinning method was used to create the nanosensor color indication pads (Figure 1) (23), then sliced into small 3 cm square pads for testing on various surfaces. All used nanosensor color indication pads were standardly packaged and sterilized before sampling and testing. After choosing the suspect or vulnerable growth surfaces for the microbes, the researcher would apply the light blue activated color indication nanosensor to the selected surfaces while wearing sterile gloves and washing his hands. Transparent and made of plastic, the adhesive covering that held the pads to the intended surface would keep moisture and water from permeating the pad's surface during sampling.

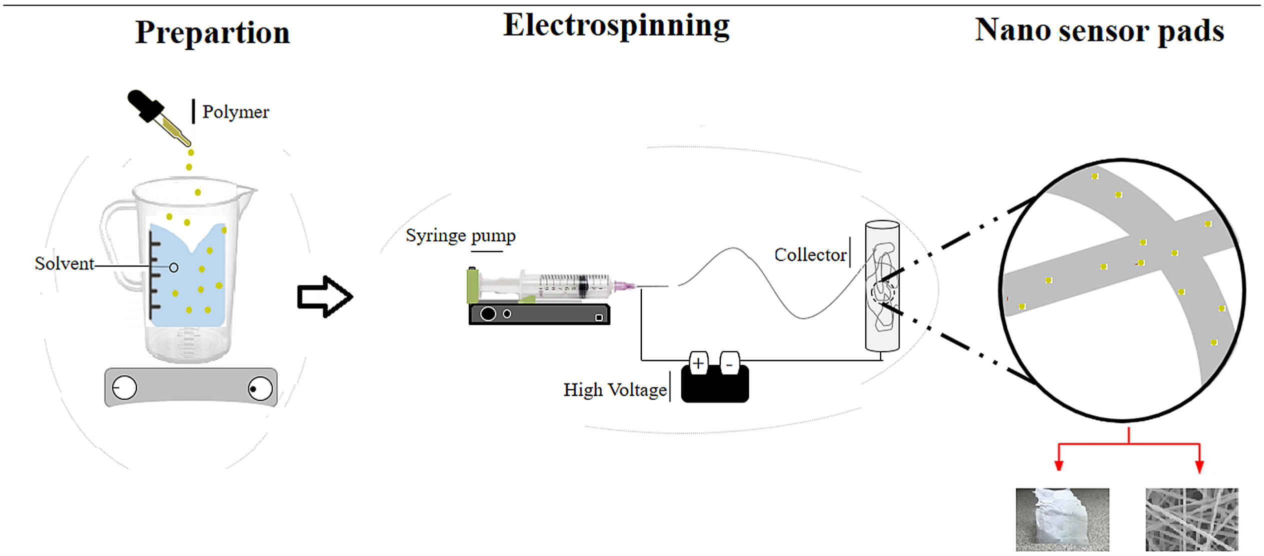

Figure 1. Fabrication of nanosensor pads using the electrospinning technique

By examining the surface of the pads and comparing their color to the color spectrum already printed on the paper, the sampler could immediately determine the breadth of the microbial load and estimate the approximate level of contamination.

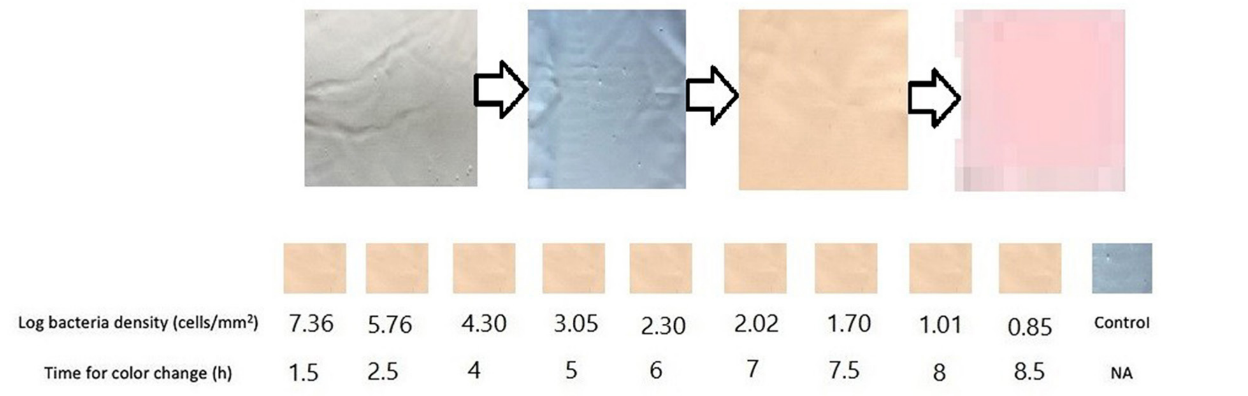

Figure 2. Changing the color of the pads according to the cells/mm2 and time-based measures of microbial contamination

By examining the surface of the pads and comparing their color to the color spectrum already printed on the paper, the sampler could immediately determine the breadth of the microbial load and estimate the approximate level of contamination.

3.2. Monitoring by Standard Microbial Culture

All equipment used was standard-packed and autoclaved sterilized before sampling and testing. We utilized class 6 indicators in every carton and sampled while wearing disposable sterile gloves to ensure sanitation. After the sterile operation, swaps were utilized to sample the selected areas and the locations of medical equipment and tools. The swap was wetted with a sterile normal saline solution to collect samples. Following this, 10 cm2 of the selected spot level was swirled in a zigzag pattern and then deposited in a test tube with 1 mL of sterile normal saline. This mixture was then mixed for ten seconds. Then, 100 microliters of the solution were cultured on plates generated in the appropriate. After 48 hours of incubation at 37°C, the growth of the bacteria on the plates was calculated and recorded using a colony counter based on CFU/cm2. Previous research found that areas with microbiological loads less than 2.5 CFU/cm2 were acceptable (clean and sanitary), while those with loads higher than 2.5 CFU/cm2 were contaminated (39, 40).

4. Results

The results of Table 1 show that the samples obtained from the standard microbial culture, ventilator, and bedside railings of the patient 50%, the oxygen manometer and Infusion pump 47.5% contamination, and the samples obtained from the nano sensors color indicator, bed side railings 55%, pulse oximetry 52.5% and Ventilator with 50% were the sources of the majority of contamination.

Table 1. Percentage of Contaminated Instances on Each Item of Selected Medical Equipment, Sorted by Frequency

Type of Equipment		Standard Microbial Cultu,re No. (%)	Nanosensor, No. (%)
1	**Cardiac monitor**	14 (35)	18 (45)
2	**Oxygen manometer**	19 (47.5)	14 (35)
3	**Suction**	16 (40)	17 (42.5)
4	**Electrocardiograph**	19 (47.5)	17 (42.5)
5	**Blood pressure cuff**	17 (42.5)	18 (45)
6	**Ventilator**	20 (50)	20 (50)
7	**Pulse oximetry**	17 (42.5)	21 (52.5)
8	**Infusion pump**	19 (47.5)	17 (42.5)
9	**Bed Side railings**	20 (50)	22 (55)
10	**Digital thermometer**	15 (37.5)	18 (45)
Total/average		176 (44)	182 (45.5)

The results of Table 2 show that salmonella, *Staphylococcus aureus,* and *Escherichia coli* had the highest prevalence, and *Acinetobacter, Enterococci*, and *Streptococcus bovis* have the lowest microbial growth rates. In this research, we refrained from writing the names of microorganisms that have grown less than 5%.

Table 2. Bacterial Frequency Per 176 Positive Cultures Obtained from 400 Cases of Culture Performed by Bacterial Separation

Bacteria Type	Total Number of Cases of Growth (%)
Streptococcus bovis	24 (13.6)
Salmonella typhimurium	42 (23.8)
Escherichia coli	98 (55.6)
Pseudomonas aeruginosa	37 (21)
Klebsiella pneumoneae	30 (17)
Staphylococcus aureus	51 (28.9)
Enterococcus faecalis	14 (7.9)
Acinetobacter baumannii	22 (12.5)

5. Discussion

The findings showed that an average of 66% of samples obtained using the nanosensor and 54.5% of samples obtained using the culture medium were clean, and the rest of the samples were contaminated, indicating a relatively high level of surface contamination of medical equipment used in ICU, and that this level of contamination is expected due to the non-sterility of the surfaces and the high accuracy and sensitivity of both methods. In their study, Ekrami et al. investigated surface contamination of medical equipment using a nano-biosensor color indicator. They found that surface contamination was present in 55% of the samples before disinfection, and after routine cleaning of the ICU ward, it decreased to 36.5%. (23). This result is consistent with the current research results in terms of the type of contamination control method and the results and confirms the findings of this research.

The findings of Malik et al., which used two methods of visual and microbial culture in four British hospitals, show that 90% of the samples in the graphical method were clean. In contrast, 90% of the samples in the microbial culture method were reported to be infected and at risk of spreading hospital infections (41). Additionally, Cooper et al. from England said that 15% of the samples obtained using the visual method and 76% of the samples obtained using the standard microbial culture medium were positive, indicating the high level of contamination reported using the standard microbial culture method (42). Comparing these results to the current study, they mean a higher degree of contamination.

In their investigation of the degree of microbial contamination of medical equipment at Kashan hospitals, Nazeri et al. discovered that 76% of the study regions were infected due to high contamination levels (43); Karami et al. used the observation method (ICNA) and the microbial method (ACC) to measure the surface contamination of medical equipment in their research. According to the ICNA approach, 61% and 39.5% of all samples before and after the routine cleaning of the ward, and according to the ACC method, 61% and 39.5% of all samples before and after the routine cleaning of the ward were contaminated (38), and Najafi Saleh et al.'s study, "assessment of ICU medical equipment levels at Neyshabur hospitals using ICNA and ACC method," found that the most and least contaminated spots, respectively, were electroshock with 1% and bottle suction with 8.2% in the ICNA method and average contamination rates of 17.43% and 78.69% in the ACC method (44). Additionally, Yosefi et al. found in their study that 47.1% (128 samples) of the samples tested positive using the microbial culture method (45). These findings support the findings of the current research in terms of the high level of contamination, research environment, and methodology and are in line with the results of the current study. According to the findings in Table 2, *E. coli,* which grew in 55.6% of the samples, and *Staphylococcus aureus,* which grew in 51% of the samples, are the most common microorganisms. Yosefi et al. (45), in their research, reported that *Staphylococcus epidermidis* and *Staphylococcus saprophyticus, Acinetobacter, Enterobacter,* and *Pseudomonas* spp are the most grown microorganisms and Najafi Saleh and al. (44) said that the microorganism of Staphylococcus epidermises is the most grown organism in the ICU.

5.1. Conclusions

Based on the results, the amount of bacterial contamination detected by each of the two tools is within the expected and acceptable range, and the results are close to each other, so considering the specific characteristics of each device, it cannot be said which tools have better efficiency. In future research, it is recommended to measure the sensitivity of these two tools in the two stages before and after routine cleaning.

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Authors' Contribution:

Ali Ekrami wrote the manuscript with support from Mohammad Ali Hosseini. Hassan Ekrami was responsible for paraphrasing the article. Hassan Ekrami fabricated the sample. Mohammad Ali Hosseini helped supervise the project. Ali Ekrami and Mohammad Ali Hosseini conceived the original idea and Mohammad Ali Hosseini supervised the project

References

1. Mosadeghrad AM, Sadeghi M. [Medical tourism: Reasons for choosing Iran]. *Payesh.* 2021;**20**(2):145-66. Persian. https://doi.org/10.52547/payesh.20.2.145.
2. Bharmal A, Ng C, Vijh R. COVID-19 Prevention Assessments: A Promising Tool for Preventing Outbreaks in Long-Term Care Homes. *J Am Med Dir Assoc.* 2021;**22**(10):2032-3. [PubMed ID:34487688]. [PubMed Central ID:PMC8372522]. https://doi.org/10.1016/j.jamda.2021.08.008.
3. Mosadeghrad AM, Qazanfari F, Keykhani S. [Hospital Infection Control accreditation standards: A Comparative Review]. *J Health Saf Work.* 2022;**12**(1):99-122. Persian.
4. Bereket W, Hemalatha K, Getenet B, Wondwossen T, Solomon A, Zeynudin A, et al. Update on bacterial nosocomial infections. *Eur Rev Med Pharmacol Sci.* 2012;**16**(8):1039-44. [PubMed ID:22913154].
5. Irwandi I, Fitriani AD, Harahap J. Implementation Analysis of Prevention and Infection Control in Datu Beru Takengon Hospital. *Journal La Medihealtico.* 2022;**3**(4):269-78. https://doi.org/10.37899/journallamedihealtico.v3i4.688.
6. Carling PC. Health Care Environmental Hygiene: New Insights and Centers for Disease Control and Prevention Guidance. *Infect Dis Clin North Am.* 2021;**35**(3):609-29. [PubMed ID:34362536]. https://doi.org/10.1016/j.idc.2021.04.005.
7. Molina Garcia A, Cross JH, Fitchett EJA, Kawaza K, Okomo U, Spotswood NE, et al. Infection prevention and care bundles addressing health care-associated infections in neonatal care in low-middle income countries: a scoping review. *EClinicalMedicine.* 2022;**44**:101259. [PubMed ID:35059614]. [PubMed Central ID:PMC8760419]. https://doi.org/10.1016/j.eclinm.2021.101259.
8. Rosenthal VD, Myatra SN, Divatia JV, Biswas S, Shrivastava A, Al-Ruzzieh MA, et al. The impact of COVID-19 on health care-associated infections in intensive care units in low- and middle-income countries: International Nosocomial Infection Control Consortium (INICC) findings. *Int J Infect Dis.* 2022;**118**:83-8. [PubMed ID:35218928]. [PubMed Central ID:PMC8866162]. https://doi.org/10.1016/j.ijid.2022.02.041.
9. Revelas A. Healthcare - associated infections: A public health problem. *Niger Med J.* 2012;**53**(2):59-64. [PubMed ID:23271847]. [PubMed Central ID:PMC3530249]. https://doi.org/10.4103/0300-1652.103543.
10. Mamishi S, Pourakbari B, Teymuri M, Babamahmoodi A, Mahmoudi S. Management of hospital infection control in Iran: a need for implementation of multidisciplinary approach. *Osong Public Health Res Perspect.* 2014;**5**(4):179-86. [PubMed ID:25379367]. [PubMed Central ID:PMC4214997]. https://doi.org/10.1016/j.phrp.2014.06.001.
11. Drohan SE, Levin SA, Grenfell BT, Laxminarayan R. Incentivizing hospital infection control. *Proc Natl Acad Sci U S A.* 2019;**116**(13):6221-5. [PubMed ID:30858309]. [PubMed Central ID:PMC6442548]. https://doi.org/10.1073/pnas.1812231116.
12. Khan HA, Ahmad A, Mehboob R. Nosocomial infections and their control strategies. *Asian Pac J Trop Biomed.* 2015;**5**(7):509-14. https://doi.org/10.1016/j.apjtb.2015.05.001.
13. Haghparast H. [Socio-Economic Burden of Hospital Acquired Infection (HAI) Translated in Persian By Hassan Haghparast]. *Journal of Health Administration.* 2002;**5**(12-13):77-82.
14. Abbasi Farid G, Bagheri Moghaddam A, Bojdy A. Nosocomial Pneumonia in Patients Admitted to the Intensive Care Unit of a Tertiary Care Center in Mashhad, Northeast of Iran; an Etiologic Survey. *Arch Clin Infect Dis.* 2018;**13**(4):e64239. https://doi.org/10.5812/archcid.64239.
15. Kollef MH, Torres A, Shorr AF, Martin-Loeches I, Micek ST. Nosocomial Infection. *Crit Care Med.* 2021;**49**(2):169-87. [PubMed ID:33438970]. https://doi.org/10.1097/CCM.0000000000004783.
16. Haque M, Sartelli M, McKimm J, Abu Bakar M. Catheter-Associated Urinary Tract Infections & Health Care-Associated Infections (HAI) - Brief Review. *World J Case Rep Clin Img.* 2022;**1**(1):1-8.
17. Okafor RA, Nyenke CU, Onosakponome EO. Health Care Associated Infections. *J Adv Microbiol.* 2022;**22**(9):104-15. https://doi.org/10.9734/jamb/2022/v22i930496.
18. Dancer SJ. The role of environmental cleaning in the control of hospital-acquired infection. *J Hosp Infect.* 2009;**73**(4):378-85. [PubMed ID:19726106]. https://doi.org/10.1016/j.jhin.2009.03.030.
19. Tomczyk S, Twyman A, de Kraker MEA, Coutinho Rehse AP, Tartari E, Toledo JP, et al. The first WHO global survey on infection prevention and control in health-care facilities. *Lancet Infect Dis.* 2022;**22**(6):845-56. [PubMed ID:35202599]. [PubMed Central ID:PMC9132775]. https://doi.org/10.1016/S1473-3099(21)00809-4.
20. Siani H, Maillard JY. Best practice in healthcare environment decontamination. *Eur J Clin Microbiol Infect Dis.* 2015;**34**(1):1-11. [PubMed ID:25060802]. https://doi.org/10.1007/s10096-014-2205-9.
21. Weber DJ, Rutala WA, Miller MB, Huslage K, Sickbert-Bennett E. Role of hospital surfaces in the transmission of emerging health care-associated pathogens: norovirus, Clostridium difficile, and Acinetobacter species. *Am J Infect Control.* 2010;**38**(5 Suppl 1):S25-33. [PubMed ID:20569853]. https://doi.org/10.1016/j.ajic.2010.04.196.
22. Ananda T, Modi A, Chakraborty I, Managuli V, Mukhopadhyay C, Mazumder N. Nosocomial Infections and Role of Nanotechnology. *Bioengineering (Basel).* 2022;**9**(2):51. [PubMed ID:35200404]. [PubMed Central ID:PMC8869428]. https://doi.org/10.3390/bioengineering9020051.
23. Ekrami A, Hosseini MA, Fallahi-Khoshknab M. Assessing the sensitivity of the Nano-Biosensor Color Indicator as a method for identifying nosocomial infection reservoirs. *Health Tech Ass Act.* 2023;**6**(3). https://doi.org/10.18502/htaa.v6i3.12430.
24. Jarvis WR. Bennett & Brachman's hospital infections. Philadelphia: Lippincott Williams & Wilkins; 2022.
25. Ekrami A, Ghadermazi M, Ekrami M, Hosseini MA, Emam-Djomeh Z, Hamidi-Moghadam R. Development and evaluation of Zhumeria majdae essential oil-loaded nanoliposome against multidrug-resistant clinical pathogens causing nosocomial infection. *J Drug Deliv Sci Technol.* 2022;**69**:103148. https://doi.org/10.1016/j.jddst.2022.103148.
26. Ekrami M, Ekrami A, Hosseini MA, Emam-Djomeh Z. Characterization and Optimization of Salep Mucilage Bionanocomposite Films Containing Allium jesdianum Boiss. Nanoliposomes for Antibacterial Food Packaging Utilization. *Molecules.* 2022;**27**(20):7032. [PubMed ID:36296625]. [PubMed Central ID:PMC9609761]. https://doi.org/10.3390/molecules27207032.
27. Ekrami M, Roshani-Dehlaghi N, Ekrami A, Shakouri M, Emam-Djomeh Z. pH-Responsive Color Indicator of Saffron (Crocus sativus L.) Anthocyanin-Activated Salep Mucilage Edible Film for Real-Time Monitoring of Fish Fillet Freshness. *Chemistry.* 2022;**4**(4):1360-81. https://doi.org/10.3390/chemistry4040089.
28. Ekrami M, Ekrami A, Moghadam RH, Joolaei-Ahranjani P, Emam-Djomeh Z. Food-based Polymers for Encapsulation and Delivery of Bioactive Compounds. In: Gopi S, Balakrishnan P, Bračič M, editors. Polymer chemistry series: *Biopolymers in Nutraceuticals and Functional Foods.* Cambridge: Royal Society of Chemistry; 2022. p. 488-544.
29. Ekrami M, Ekrami A, Esmaeily R, Emam-Djomeh Z. Nanotechnology-based Formulation for Alternative Medicines and Natural Products: An Introduction with Clinical Studies. In: Gopi S, Balakrishnan P, Bračič M, editors. *Polymer chemistry series: Biopolymers in Nutraceuticals and Functional Foods.* Cambridge: Royal Society of Chemistry; 2022. p. 545-80.
30. Emam-Djomeh Z, Ekrami M, Ekrami A. Design and Use of Hydrogels for Food Component Encapsulation. In: Lai WF, editor. *Materials Science and Engineering in Food Product Development.* Hoboken, NJ: John Wiley & Sons; 2023. p. 211-26.
31. 31.Ekrami A, Hosseini MA, Ekrami H. [Comparative Evaluation of the level of contamination of internal hospital surfaces, using two methods: nano biosensor and microbial culture]. *The Journal of Tolooebehdasht.* 2023;**22**(1):47-60. Persian. https://doi.org/10.18502/tbj.v22i1.12779.
32. Shakouri M, Salami M, Lim L-T, Ekrami M, Mohammadian M,

Askari G, et al. Development of active and intelligent colorimetric biopolymer indicator: anthocyanin-loaded gelatin-basil seed gum films. *J Food Meas Charact.* 2022;**17**(1):472-84. https://doi.org/10.1007/s11694-022-01640-7.

33. Ekrami M, Emam Jomeh Z, Mirzakhani M. [Physical and mechanical properties of biodegradable edible film obtained from Salep]. *Iranian Journal of Biosystems Engineering.* 2014;**45**(1):45-51. Persian. https://doi.org/10.22059/ijbse.2014.51290.
34. Ekrami M, Babaei M, Fathi M, Abbaszadeh S, Nobakht M. Ginger essential oil (Zingiber officinale) encapsulated in nanoliposome as innovative antioxidant and antipathogenic smart sustained-release system. *Food Biosci.* 2023;**53**:102796. https://doi.org/10.1016/j.fbio.2023.102796.
35. Farahmand E, Emam-Djomeh Z, Ekrami M, Razavi SH. Polymethacrylate coated electrospun chitosan/PEO nanofibers loaded with thyme essential oil: a newfound potential for antimicrobial food packaging. *J Food Bioprocess Eng.* 2023. https://doi.org/10.22059/jfabe.2023.356018.1138.
36. Emam-Djomeh Z, Ekrami M, Ekrami A. Overview of Types of Materials Used for Food Component Encapsulation. In: Lai WF, editor. *Materials Science and Engineering in Food Product Development.* Hoboken, NJ: John Wiley & Sons; 2023. p. 73-92.
37. Schabrun S, Chipchase L. Healthcare equipment as a source of nosocomial infection: a systematic review. *J Hosp Infect.* 2006;**63**(3):239-45. [PubMed ID:16516340]. https://doi.org/10.1016/j.jhin.2005.10.013.
38. Karami G, Khazei M, Rasuli Ravandi F, Emtiyazipoor Z. [Evaluating the effect of hospital medical instruments on the nosocomial infection risk]. *Nurs Midwifery J.* 2015;**13**(7):579-87. Persian.
39. Stein M, Lipman-Arens S, Oved K, Cohen A, Bamberger E, Navon R, et al. A novel host-protein assay outperforms routine parameters for distinguishing between bacterial and viral lower respiratory tract infections. *Diagn Microbiol Infect Dis.* 2018;**90**(3):206-13. [PubMed ID:29273482]. https://doi.org/10.1016/j.diagmicrobio.2017.11.011.
40. Riyahin AA, Eshraghi M, Gharehbeglou M, Ahmadli Z, Karami G, Shahrzad ME. Evaluation of ICU microbial contamination in Qom hospitals using observational and microbial monitoring methods with three indices of observation, colony count, and methicillin-resistant S. aureus. *Journal of Current Research in Science.* 2014;**2**(6):788. https://scholar.google.com/scholar?hl=en&as_sdt=0%2C5&q=Evaluation+of+ICU+microbial+contamination+in+Qom+hospitals+using+observational+and+microbial+monitoring+methods+with+three+indices+of+observation%2C+colony+count%2C+and+methicillin-resistant+S.+a&btnG=
41. Malik RE, Cooper RA, Griffith CJ. Use of audit tools to evaluate the efficacy of cleaning systems in hospitals. *Am J Infect Control.* 2003;**31**(3):181-7. [PubMed ID:12734526]. https://doi.org/10.1067/mic.2003.34.
42. Cooper RA, Griffith CJ, Malik RE, Obee P, Looker N. Monitoring the effectiveness of cleaning in four British hospitals. *Am J Infect Control.* 2007;**35**(5):338-41. [PubMed ID:17577482]. https://doi.org/10.1016/j.ajic.2006.07.015.
43. Nazeri M, Salmani Arani J, Ziloochi N, Delkhah H, Hesami Arani M, Asgari E, et al. Microbial contamination of keyboards and electronic equipment of ICU (Intensive Care Units) in Kashan University of medical sciences and health service hospitals. *MethodsX.* 2019;**6**:666-71. [PubMed ID:30997346]. [PubMed Central ID:PMC6453683]. https://doi.org/10.1016/j.mex.2019.03.022.
44. Najafi Saleh H, Kavosi A, Pakdel M, Yousefi M, Baghal Asghari F, Mohammadi AA. Assessment health status of ICU medical equipment levels at Neyshabur hospitals using ICNA and ACC indices. *MethodsX.* 2018;**5**:1364-72. [PubMed ID:30425934]. [PubMed Central ID:PMC6222287]. https://doi.org/10.1016/j.mex.2018.10.016.
45. Yosefi M, Hasanzadeh F, Tabatabeizadeh A, Naderi H, Khadem Rezaiyan M. [Investigation of microbial contamination in air and interior surfaces of two teaching hospitals of Mashhad University of Medical Sciences]. *Journal of Research in Environmental Health.* 2021;**7**(2):133-42. Persian. https://doi.org/10.22038/jreh.2021.57152.1418.