

Assessing the sensitivity of the Nano-Biosensor Color Indicator as a method for identifying nosocomial infection reservoirs.

Ali Ekrami¹, Mohammad-Ali Hosseini^{*2}, Masoud Fallahi-Khoshknab²

¹Master's Degree in Nursing, Department of Nursing, University of Social Welfare and Rehabilitation Sciences, (USWR) Tehran, Iran .

²Professor of Nursing, University of Social Welfare and Rehabilitation Sciences (USWR) Tehran, Iran.

Corresponding Author: Mohammad Ali Hosseini, Department of Nursing, University of Social Welfare and Rehabilitation Sciences, Tehran, Iran. *Email: Ma.Hosseini@USWR.AC.IR
Tel:02122180083

Received 2022 April 01; Accepted 2022 April 28.

Abstract

Groundbreaking knowledge at the nanoscale has been developed in many functional and industrial situations. Nanoscale colour recognition sensors have been developed by researchers in order to offer new ways to identify harmful germs. This study objective was to evaluate the ability of nano-biosensors colour indicators to identify nosocomial infection reservoirs.

Method: The enquiry was conducted in a semi-experimental environment for a month. Samples were obtained both prior to and during standard disinfection. The final findings were described as both clean and infected. There were 400 cases in the sample, 10 different types of equipment were employed, and sampling was done.

Results: The results showed high contamination of the studied surfaces. Moreover, 55% of the samples were infected before disinfection, which was reduced to 36.5% after routine disinfection.

Conclusion: The findings show that this tool has the necessary sensitivity, and we can introduce it as a standard tool for microbial contamination in the future; however, the aim was not to identify the type of microorganism.

Keywords: Infection Control, Nanotechnology, Indicators and Reagents, Healthcare Associated Infections, Cross Infection

1. Introduction

Today, one of the significant challenges of the country's healthcare system is the incidence of nosocomial infections. Nosocomial infections are essential in terms of infection, attenuation, and cost. In addition to mortality and complications, these infections, by increasing the length of hospital stay, significantly increase the cost of healthcare and ultimately cause dissatisfaction among recipients (1, 2). Each year, these infections have a heavy financial burden on the secondary, tertiary, and primary healthcare systems, the patients, and those who provide care for them (3-5). Correct diagnosis and timely treatment of hospital infections reduce hospital infections and the effects of imposing false treatment costs on patients and society. Therefore, hospitals should develop an infection control program to assess and control the prevalence of infections (6, 7).

Despite many advances in infection control, many methods used face limitations, including difficulty in prototyping and time-consuming. Today, various sensors are used to solve the problems of other methods, such as time-consuming and the need for extensive laboratory

equipment. Sensors that detect pathogens and dense and compact structures must be sensitive, specific, and fast to be used even in remote and rural areas (8, 9). According to the mentioned features, researchers use new methods using the unique properties of nanomaterials in the design of various sensors to identify and measure environmental pollution (9-11). Nanotechnology significantly impacts environmental research, transportation, energy, food safety, information technology, and medicine, among other fields and industries. Nanotechnology primarily provides illness prevention, diagnosis, and therapy solutions in medicine. Biosensors are part of a technical innovation that has exploded to better understand highly complex and dynamic biological processes. Although biosensors were recognized as early as 1962, combining biosensors with nanotechnology has given medicine a new dimension (12, 13). According to the most commonly recognized definition, a biosensor is an analytical device that incorporates a biologically active element with an appropriate physical transducer to provide a quantifiable signal proportional to the concentration



of chemical species in any sample type (14-17). Biosensors are categorized based on their biological signaling mechanism or the signal transduction method (17, 18).

Despite their many benefits, the use of new health technologies has consequences and has posed some challenges to health systems. Therefore, emerging health technologies must be appropriately evaluated and used efficiently and effectively for healthcare. For this purpose, many countries worldwide have tried to devise mechanisms for the rational entry and use of these technologies to control costs on the one hand and prevent the import of technologies that are low in safety and effectiveness on the other hand. One of the most important methods for this purpose, common in many developed countries, is health technology evaluation (8, 19). Health technology assessment is an interdisciplinary practice that systematically examines the technical performance, safety, clinical effectiveness, cost-effectiveness, organizational implications, social consequences, and legal and ethical considerations of health technology (20). Evaluating health technology, as a critical decision-making and prioritization tool, is still a new concept in most developing countries, but it is expanding rapidly. The World Health Organization (WHO) has also emphasized evaluating health technology for over two decades (8, 21).

In Iran, valuable measures have been taken in health technology evaluation in recent years. Evidence in some other countries, including India, shows that most health technology projects in this country are based on equipment technologies, but new projects in this country and equipment are moving toward new diagnostic methods. Furthermore, screening has been driven (8, 21, 22).

New technologies, including nanotechnology, offer an excellent opportunity to develop rapid, new, sensitive, proprietary, and cost-effective techniques for detecting pathogenic microorganisms. Therefore, the development of rapid, sensitive, and highly specific (proprietary) methods based on nanotechnology to identify pathogenic bacteria has been at the forefront of community health control research. Researchers have developed biosensors as beneficial and practical equipment for accurate and rapid identification in recent years. It is expected that this new strategy will have the ability to develop and introduce a dedicated platform for the detection of a multiplex of multiple bacterial species. This new technology can be an excellent alternative to specialized or cost-effective methods if proven effective in introducing the existing tradition (23).

Nosocomial infections, commonly known as health-care-associated infections (HAIs), are illnesses acquired while undergoing medical treatment but not present at admission. They can emerge in various settings, including hospitals, long-term care facilities, outpatient clinics, and after discharge. Occupational infections, which may affect employees, are also considered HAIs. When a pathogen infects a patient host, infection occurs. Infection happens when a pathogen infects a patient host. In

the context of contemporary healthcare, invasive procedures, including surgery, indwelling medical equipment, and prosthetic devices, are associated with infection. The etiology of HAIs is determined by the infection's origin or kind and the causing bacterial, viral, or fungal pathogen (2, 24-26).

Nosocomial infections produced by certain microbial strains continue to cause death and morbidity worldwide significantly (27). Given that preventing nosocomial infections is a critical way to improve the quality of healthcare services and is essential in the prevention and infection of nurses and patients, accurate data on the scope of these infections is critical in evaluating current infection prevention activities. It is also necessary to plan for future intervention in hospitals, determine the cause and risk factors of nosocomial infections, and use appropriate infection control measures (28, 29). Nano biosensors are primarily used to control spoilage of food, pharmaceuticals, and chemistry and are less used in the field of healthcare and hospital. Therefore, according to the researcher's many years of experience in hospital departments as a nurse, observing and understanding the side effects of patients with hospital infections, and the researcher's previous side activity in the field of optimization and manufacturing of nano biosensors (30-34), this study was conducted to Assessing the sensitivity of the Nano-Biosensor Color Indicator as a method for identifying nosocomial infection reservoirs. The mentioned plan with the ethics code IR.USWR.REC.1399.164 has been approved by the research Vice-chancellor of Tehran University of Social Welfare and Rehabilitation Sciences.

2. Materials and methods:

This semi-experimental study was performed in intensive care units (ICU) of Imam Khomeini Hospital complex in Tehran for one month, before and after routine disinfecting of the ward, randomly twice a week. Sample volume is based on the volume calculation formula (Quackran formula online). According to the study by Karami et al. (2015), with $P = 0.025$, an acceptable error of $d = 0.043$, and a 95% confidence level, the sample size was 400. The cases were calculated as follows: 200 samples before disinfecting and 200 samples after disinfecting (35). The samples were taken from the ICU departments' medical equipment surfaces whose contamination had previously been established by research. The samples were first selected from a targeted type based on the inclusion criteria of the devices. Then, the sampling was performed by a color indicator nano biosensor. First, the researcher selected ten types of medical equipment that are most probably to have microbial contamination and are in direct and indirect contact with nurses and patients. Then, sampling was performed before and after routine cleaning.

Monitoring by color indicator nano biosensors:

Accordingly, after production of color indicator nano biosensor pads, made by Zhang's method (36) with a few modifications and fabrication of nano biosensor pads by

electrospinning method (Fig 1), they were cut into small 3 cm square pads for testing on different surfaces.

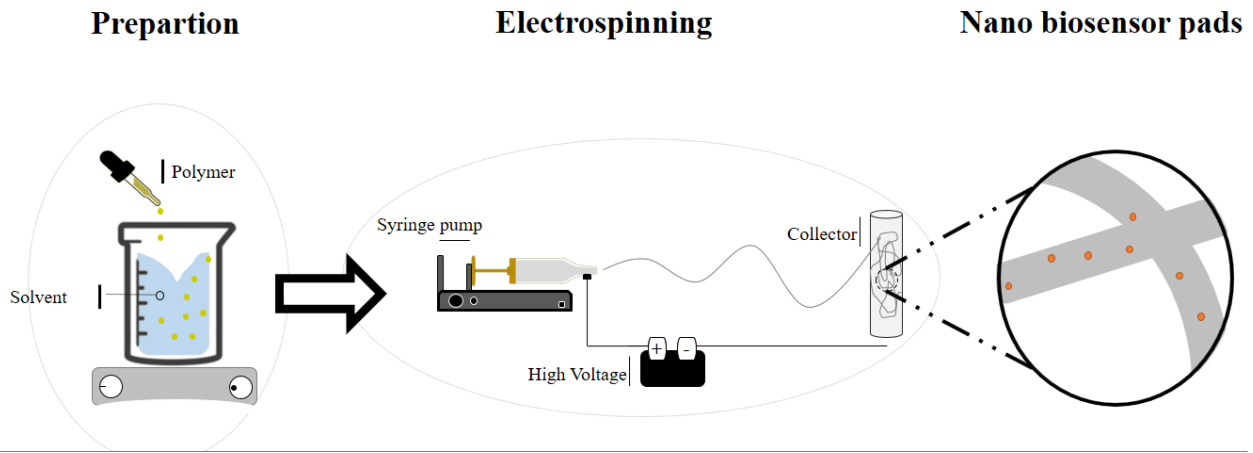


Figure 1. Fabrication of nano biosensor pads by electrospinning method.

After selecting the suspect or susceptible growth surfaces of the microorganisms. The researcher would attach the activated color indicator nano biosensor, light blue, to the desired surfaces after washing his hands and wearing sterile gloves.

The adhesive coating that connected the pads to the desired surface was transparent and plastic and would prevent water and moisture from penetrating from outside during sampling and cover the pad's surface (Fig 2). The pads usually are light blue and will have a visible color change from blue to red in the presence of microbial load (Fig 3).



Figure 2. The color recognition nano biosensor pads' ultimate shape.

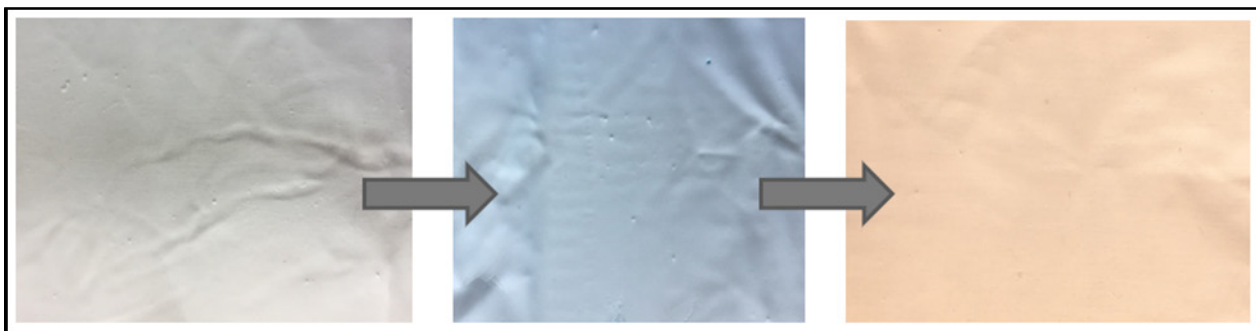


Figure 3. Nano biosensor pad's color changes when it comes into touch with bacteria contamination.

After discoloration, which might take a few hours and a few days, the color change of all pads was checked after 24 hours. The sampler could quickly assess the extent

of microbial load by simply looking at the surface of the pads and comparing its color with the color spectrum already printed on the paper, and estimating the approxi-

mate amount of contamination. According to previous studies, points with a microbial load greater than 3 CFU/cm² would change the color of the pads from light blue

to red (Fig 4)(36). The higher the microbial load, the more significant the color change (37-39).

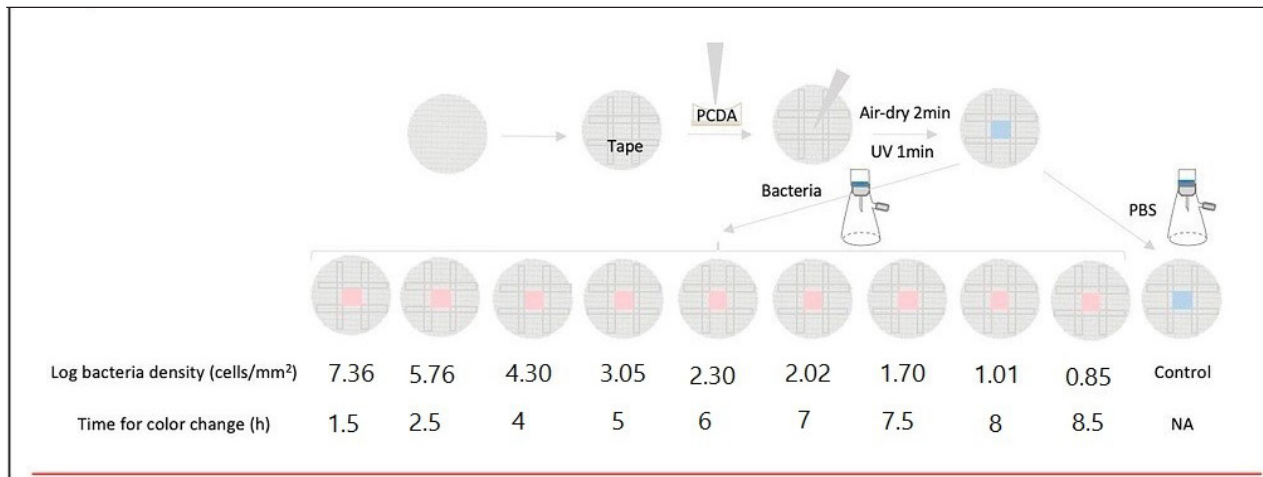


Figure 4. Changing the color of the pads based on the amount of microbial contamination based on cells/mm² and time.

3. RESULTS:

The result of the Kolmogorov-Smirnov test on the data shows that the distribution of the data obtained by the

culture medium method, which was done before and after disinfecting, does not follow the normal distribution (Sig <0.05). Therefore, in this study for analysis and Statistical analysis will use non-parametric tests.

Table 1. Frequency distribution of the number of Contaminated cases on each of the selected medical equipment

	Type of equipment	After Disinfection		Before Disinfection	
		Percent	Contaminated	Percent	Contaminated
1	Cardiac monitor	25%	5	65%	13
2	Oxygen manometer	30%	6	40%	8
3	Suction	30%	6	55%	11
4	Electrocardiograph	40%	8	45%	9
5	Blood pressure cuff	35%	7	55%	11
6	Ventilator	50%	10	50%	10
7	Pulse oximetry	45%	9	60%	12
8	Infusion pump	35%	6	55%	11
9	Bed Side	35%	7	75%	15
10	Digital thermometer	40%	8	50%	10
Total / Average		36.5%	72	55%	110

According to Table 1, the contamination of the studied surfaces was high. On average, 55% of the studied surfaces were contaminated, which decreased to 36.5% after routine disinfection. Before routine disinfection, the

bedside, cardiac monitoring, and pulse oximetry had the highest rates of contamination (75%, 65%, and 60%, respectively), while after disinfection, these rates dropped to 35%, 25%, and 45%, respectively.

Table 2. Evaluation of the sensitivity of color indicator nano biosensor

Chi-square	Infected	Percent	Clean	Percent	
Sig: 0.00	110	55%	90	45%	Before disinfection
	72	36%	128	64%	After disinfection

The Chi-square test was used to evaluate the sensitivity of the color indicator nano biosensor (Table 2). In this re-

search, the sensitivity of the tool means that the tool has the ability to detect the change in contamination level

(decrease/increase) after cleaning. According to Table 2, the amount of contamination measured by the nano biosensor was related to routine cleaning ($P < 0.05$), so that after cleaning, on average, we saw an 18.5% reduction in contamination.

4. Discussion:

According to this study, the prevalence of contaminated locations with significant microbial load was relatively high in medical equipment in the ICU. However, following frequent cleaning, the contamination significantly decreased (18.5%), suggesting a high diagnostic power color indicator nano biosensor. Considering the high sensitivity of the nano biosensors in detecting contamination and the maximum time frame for color change (8 hours), an 18.5% reduction in contamination after routine cleaning seems reasonable. Our findings demonstrated the diagnostic sensitivity of this tool to reduce microbial contamination and this tool potentially has the necessary sensitivity.

Mehdi Nazari et al. (2019) studied the microbial contamination of medical equipment in Kashan hospitals (40) and reported high contamination in the study areas, so 76% of the study areas were infected. Karami et al. (2015) used two instruments, Microbial Method (ACC^1) and Observation Method ($ICNA^2$), to measure the surface contamination of medical equipment in their study (35). According to the ICNA approach, 61% and 39.5% of all objects were unclean before and after the interventions. According to the ACC approach, 76% of all objects were dirty before the intervention, and 69.5% were filthy afterward. The findings support our findings of the high degree of contamination on medical equipment surfaces and the subsequent reduction in contamination following cleaning. Yousefi et al. (2021), in their research entitled "Investigation of Microbial Contamination in the Environment and Medical Equipment of Ghaem Hospital of Mashhad" reported that a convenience sampling method was used based on the policies of the infection control committee of this hospital. Environmental samples were collected from the sink, patient bed, and incubators. Of 272 samples obtained by microbial culture method, 47.1% (128 samples) were positive ($p = 0.071$). Also, Yousefi et al., in another study that measured the level of surface contamination of the medical equipment of Imam Reza Hospital in Mashhad with the same method, reported that 17 out of 51 samples (33.3%) were contaminated (41). The number of cases of infection diagnosed is close to the number of cases diagnosed with the infection control tool in the current research. It shows that color indicator nano biosensor has the appropriate diagnostic capability. In a study in Qom in 2014, Riahi et al. used observational and microbiological culture methodologies to compare the health state of

¹Aerobic colony count

²Aerobic colony count

ambient surfaces in ICU before and after daily cleaning. In this investigation, nine more likely-to-be-contaminated locations were chosen for sampling and were observed for ten weeks. The findings showed that the percentage of contaminated points using the observational approach before and after the cleaning was 59% and 41.5%, respectively. Both of these percentages were 57.5% and 47.5% in the microbiological method. The findings of this study closely resembled those of our study and agreed with those results (42). Najafi Saleh et al.'s study (2018) entitled "Assessment of ICU medical equipment levels at Neyshabur hospitals using ICNA and ACC indices" reported that 826 ICNA checklists were completed in this research for the 13 studied spots. Altogether, 27.12% of the spots were contaminated before cleaning procedures, which dropped to 7.75% after cleaning. The data of the samples, using ACC index, revealed that 74.82% of the spots were contaminated before cleaning procedures, dropping to 7.75% after cleaning. Bottle suction had the most contamination in the before and after samples (43), and the results obtained with this contamination control tool are close to the results obtained with color indicator nano biosensor and are in line with it. Therefore, it can be concluded that the nano biosensor has an acceptable performance in detecting contaminated areas. In future research, it is also suggested to evaluate the performance of these functional nano biosensors on a larger scale in other hospital departments, such as the operating rooms, maternity ward, laboratory, etc. Also, researchers can optimize these tools to detect a specific type of bacteria.

5. Conclusions:

After reducing microbial contamination, our research investigated the diagnostic sensitivity of this tool. The results indicated that this tool has the required sensitivity, so in cases where the goal is rapid identification and the separation of the type of microorganisms is not considered, we can introduce it as a standard tool for controlling microbial contamination; Health policymakers and hospital managers can use this helpful tool as an alternative to routine infection control methods. Among the advantages of this tool, the following can be mentioned: it is easy to use, its sensitivity is high for detecting contamination, it does not require complicated tools and equipment, it does not require expert personnel to read the samples, it has a high response rate, and its production is not expensive.

Conflict of interest

The authors of this article declare that they have no conflict of interest.

Acknowledgments

The authors would like to thank the officials of Tehran University of Welfare and Rehabilitation Sciences, Department of Food Industry, University of Tehran, and the respected officials of Imam Khomeini Hospital in Tehran for their comprehensive support of this study.

References

- Mamishi S, Pourakbari B, Teymuri M, Babamahmoodi A, Mahmoudi S. Management of hospital infection control in Iran: a need for implementation of a multidisciplinary approach. *Osong public health and research perspectives*. 2014;5(4):179-86.
- Drohan SE, Levin SA, Grenfell BT, Laxminarayan R. Incentivizing hospital infection control. *Proceedings of the National Academy of Sciences*. 2019;116(13):6221-5.
- Haghparsat H. Socio-Economic Burden of Hospital Acquired Infection (HAI) Translated in Persian. *Journal of Health Administration*. 2002;5(13):76-82.
- Pour Reza A. Hospital infection and healthcare economics. *Journal of Health Administration*. 1998;2(3):137-50.
- Ismaili SM, Yousefi M, Taghipour A, Naderi H, Aelami MH, Eftekharpour E. Antibiotic use and cost in nosocomial Pneumonia infection: Evidence from Intensive care units of a large hospital. *Medical Journal of Mashhad university of medical sciences*. 2021;63(6).
- Lu D, Wang H, Yu R, Yang H, Zhao Y. Integrated infection control strategy to minimize nosocomial infection of coronavirus disease 2019 among ENT healthcare workers. *Journal of Hospital Infection*. 2020;104(4):454-5.
- Moazeni M, Asgari S, Nabili M. Nosocomial fungal infections: Epidemiology, diagnosis, treatment, and prevention. *Journal of Mazandaran University of Medical Sciences*. 2018;28(160):182-212.
- Boos N. *Health care technology assessment and transfer*. Springer; 2007. p. 1291-2.
- Naidu Krishna S, Govender P, Adam JK. Nanosilver particles in biomedical and clinical applications. *Journal of pure and applied microbiology (Print)*. 2015.
- Martínez JL. Antibiotics and antibiotic resistance genes in natural environments. *Science*. 2008;321(5887):365-7.
- Kollef MH, Torres A, Shorr AF, Martin-Loeches I, Micek ST. Nosocomial infection. *Critical care medicine*. 2021;49(2):169-87.
- Solaimuthu A, Vijayan AN, Murali P, Korrapati PS. Nano-biosensors and their relevance in tissue engineering. *Current Opinion in Biomedical Engineering*. 2020;13:84-93.
- Afsharipour M, Mahmoudi S, Raji H, Pourakbari B, Mamishi S. Three-year evaluation of the nosocomial infections in pediatrics: bacterial and fungal profile and antimicrobial resistance pattern. *Annals of Clinical Microbiology and Antimicrobials*. 2022;21(1):1-7.
- Arnold MA, Meyerhoff ME. Recent advances in the development and analytical applications of biosensing probes. *Critical Reviews in Analytical Chemistry*. 1988;20(3):149-96.
- Eggins BR. *Chemical sensors and biosensors*: John Wiley & Sons; 2002.
- Wilson GS, Gifford R. Biosensors for real-time in vivo measurements. *Biosensors and Bioelectronics*. 2005;20(12):2388-403.
- Touhami A. Biosensors and nanobiosensors: design and applications. *Nanomedicine*. 2014;15:374-403.
- Bhattarai P, Hameed S. Basics of biosensors and nanobiosensors. *Nanobiosensors: From Design to Applications*. 2020:1-22.
- Dehnavieh R, Rashidian A, Maleki MR. Challenges of determining basic health insurance package in Iran. *Payesh (Health Monitor)*. 2011;10(2):273-83.
- Busse R, Orvain J, Velasco M, Perth M, Drummond M, Jørgensen T, et al. Best practice in undertaking and reporting health technology assessments: Working Group 4 report. *International Journal of technology assessment in health care*. 2002;18(2):361-422.
- Goodman CS. HTA 101 Introduction to health technology assessment 2014.
- Goodman CS. Introduction to health technology assessment. The Lewin Group Virginia, USA. 2004.
- Xu L, Shoaie N, Jahanpeyma F, Zhao J, Azimzadeh M, Al KT. Optical, electrochemical and electrical (nano) biosensors for detecting exosomes: A comprehensive overview. *Biosensors and Bioelectronics*. 2020;161:112222.
- Sikora A, Zahra F. Nosocomial infections. *StatPearls [Internet]*. 2021.
- Mispah CP, Kelamane S. Nosocomial infections in Neonatal Intensive Care Unit (NICU) at a Tertiary Care Hospital, Karimnagar, Telangana. 2021.
- Parthasarathy A, Menon P, Nair M. *IAP Textbook of pediatrics*: Jaypee Brothers Medical Publishers; 2019.
- Jenkins DR. Nosocomial infections and infection control. *Medicine*. 2017;45(10):629-33.
- Lu D, Wang H, Yu R, Yang H, Zhao Y. Integrated infection control strategy to minimize nosocomial infection of coronavirus disease 2019 among ENT healthcare workers. *The Journal of hospital infection*. 2020;104(4):454.
- Moosazadeh M, Eybpoosh S, Nasirian M, Molavi Vardanjani H, Sheikhzadeh K, Afshari M, et al. Explanation of the status of nosocomial surveillance system in Iran: A Qualitative Study. *Journal of Qualitative Research in Health Sciences*. 2020;4(4):406-25.
- Ekrami A, Ghadermazi M, Ekrami M, Hosseini MA, Emam-Djomeh Z, Hamidi-Moghadam R. Development and evaluation of Zhermeria majdae essential oil-loaded nanoliposome against multi-drug-resistant clinical pathogens causing nosocomial infection. *Journal of Drug Delivery Science and Technology*. 2022;69:103148.
- 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.
- Ekrami M, Ekrami A, Moghadam RH, Joolaei-Ahranjani P, Emam-Djomeh Z. Food-based Polymers for Encapsulation and Delivery of Bioactive Compounds. *Biopolymers in Nutraceuticals and Functional Foods: Royal Society of Chemistry*; 2022. p. 488-544.
- Ekrami M, Ekrami A, Esmaeily R, Emam-Djomeh Z. Nanotechnology-based Formulation for Alternative Medicines and Natural Products: An Introduction with Clinical Studies. *Biopolymers in Nutraceuticals and Functional Foods*. 2022:545-80.
- 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.
- Karami G, Khazaei M, RASULI RF, EMTIYAZIPOOR Z. Evaluating the effect of hospital medical instruments on the nosocomial infection risk. 2015.
- Zhang Y, Dawson PL, Hanks TW, Northcutt JK, Tzeng T-R, Pennington WT. Detecting and correlating bacterial populations to the visual color change of polydiacetylene-coated filters. *Talanta*. 2021;221:121482.
- Silbert L, Ben Shlush I, Israel E, Porgador A, Kolusheva S, Jelinek R. Rapid chromatic detection of bacteria using a new biomimetic polymer sensor. *Applied and environmental microbiology*. 2006;72(11):7339-44.
- Meir D, Silbert L, Volinsky R, Kolusheva S, Weiser I, Jelinek R. Colorimetric/fluorescent bacterial sensing by agarose-embedded lipid/polydiacetylene films. *Journal of applied microbiology*. 2008;104(3):787-95.
- Ma G, Cheng Q. Vesicular polydiacetylene sensor for colorimetric signaling of bacterial pore-forming toxin. *Langmuir*. 2005;21(14):6123-6.
- Nazeri M, Arani JS, Ziloochi N, Delkhah H, Arani MH, 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.
- Yosefi M, Hasanzadeh F, Tabatabaeizadeh A, Naderi H, Khadem Rezaian M. Investigation of microbial contamination in the 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.
- Riyahin AA, Eshraghi M, Gharehbeiglou 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.
- Saleh HN, Kavosi A, Pakdel M, Yousefi M, Asghari FB, Mohammadi AA. Assessment of health status of ICU medical equipment levels at Neyshabur hospitals using ICNA and ACC indices. *MethodsX*. 2018;5:1364-72.