

Evaluation of Bacterial Contaminants on N95 FFRs after Reuse in Hospital Personnels: A Prospective Study in the Era of COVID-19 Pandemic

REKHA SINGH¹, ARPANA SINGHAL², MOHAMMED ABBAS ALI³, TASNEEM ZAHRA⁴, ANUP K SAINI⁵, GAURAV DALELA⁶

ABSTRACT

Introduction: N95 Filtering Face-piece Respirators (FFRs) prevent the spread of Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) infection and protect medical personnel. It is considered as one of the essential protective equipment. An increased demand for N95 FFRs during the Coronavirus Disease 2019 (COVID-19) pandemic has resulted in shortage crisis for the care of cases arising in hospitals. Therefore, this situation generated the need to implement alternatives like four mask policy that allows reuse of N95 FFRs in settings with limited resources.

Aim: To find out the burden of bacterial isolates on N95 FFRs after reuse.

Materials and Methods: The present study was a prospective study conducted at Government RDBP Jaipuria Hospital, Jaipur, Rajasthan, India, from May 2021 to October 2021

on 526 used N95 FFRs after multiple or extended use by 96 hospital personnel who attended Outpatient Department (OPD) services. Swab samples were collected from both external, as well as, internal surface of the mask after each use and further subcultured to identify any microorganism present on them.

Results: Growth of gram positive bacilli, and micrococci were found on 362 samples out of total 526 samples. Other bacteria like *Pseudomonas* spp. and other gram negative were also observed in 19 samples. Furthermore, it was also observed that bacterial load on reused N95 FFRs was mainly commensal flora.

Conclusion: Mainly commensal floras were found over internal and external surface of used N95 FFRs. This proves that four mask policy is optimum for judicious use of N95 FFRs and resulted in saving of funds for other more emergency works mainly in OPD settings.

Keywords: Commensal flora, Coronavirus disease-2019, Four mask policy

INTRODUCTION

As respiratory viruses require protection from aerosol entry, there is need of protection against it by making a policy against transmission [1]. COVID-19 is a contagious disease caused by SARS-CoV-2 virus, seventh human coronavirus identified. In December 2019, the first known case of COVID-19 was identified in Wuhan, China [2]. The disease has since spread worldwide, leading to an ongoing pandemic which is still going on. In India, first case of COVID-19 was reported in Kerala on 27th January 2020.

Regular increase in COVID-19 cases led to increase in the demand of medical resources for personal protection and N95 FFRs, so judicious use of N95 FFRs and other medical resources is important in the era of pandemic [3]. Reuse of respirators indicates use of same N95 respirator multiple times but doffing after each encounter and keeping in storage before next use ('donning'). For pathogens without contact transmission (e.g., fomites) reuse has been practiced [4]. Extended use indicates use of same N95 respirator without removing while handling many close contact encounters with several patients. Extended use may be of utmost importance where patients are infected with the same pathogen of respiratory origin in hospital setting. It is method of conservation of respirators during an outbreak, epidemic or pandemic [5,6].

Respiratory pathogens i.e., present on the respirator surface can be transferred by touching self or someone directly or indirectly [7-9]. In some studies, these can be infectious on the surface of respirator for a long time, in microbial transfer [10-12] and in re-aerosolisation [13-16]. More than ~99.8% has remained trapped on the respirator after handling or following simulated cough or sneeze.

Frequent handwashing can be a measure to reduce the risk of viral transmission by 55% [17]. N95 FFRs could block 99.98% of viruses in aerosols [18]. N95 FFRs (N95 refers to a National Institute for Occupational Safety and Health (NIOSH)-approved) [19] was considered as an essential protective equipment while handling patients with COVID-19. Therefore, wearing N95 mask is very important mode of preventing the aerosol spread of COVID-19.

Infectious material re-aerosolisation is not found under normal use conditions, but infectious material deposited on a respirator can be a vehicle for direct or indirect transmission. Therefore, additional infection control practices are needed. Decontamination of N95 FFRs by steam sterilisation methods like humid heat with autoclaves, pressure cookers, or microwavable steam bags, disinfectants (e.g., bleach, hydrogen peroxide vapour), or Ultraviolet Germicidal Irradiation (UVGI) may be safe and effective in some settings [3].

The problem of insufficient supply of N95 FFRs in the hospitals during COVID-19 pandemic, generated the need to implement alternatives that allowed the reuse of N95 masks. Single-use N95 respirators were critical to protect the staff and patients from airborne infections, but shortages during the crisis compelled the reuse of N95 masks. The potential risks and benefits of these practices may vary greatly across locations and may evolve rapidly during a crisis. The present study was intended to provide practical guidance on the potential risks and benefits that clinical centres should consider during decision making about N95 respirator reuse.

MATERIALS AND METHODS

The present study was a prospective study conducted in Government RDBP Jaipuria Hospital, Jaipur, Rajasthan, India from May 2021 to

October 2021. Ethical approval was taken from institution, IEC approval no Letter no./office /Ethics Comm./P-26/2020 Dated 9/11/2020.

Study Procedure

A total of 263 (from which 526 samples both from outer and inner surface were taken) used N95 FFRs after reuse by 96 hospital personnel (44 Doctors, 26 nursing staff, 14 paramedical staff, and 6 ward boys and 6 ward ladies) were used as study samples. Swab samples were collected randomly from external and internal surface of the mask during random cycle of used N95 FFRs. Hospital personnel had followed guidelines for extended use of N95 FFRs issued on 08.04.2020 by All India Institute of Medical Sciences, New Delhi, India [19].

Every healthcare worker was provided with five N95 masks and four small brown covers numbered 1, 2, 3 and 4 as well as a large brown cover. Each N95 mask was placed in separate small paper bags and both the mask and the bag were labeled as 1, 2, 3 and 4. Fifth mask was issued as a reserve. On day 1st, one was supposed to wear the mask no. 1 when stepping out for duty. After returning home, one had to place the N95 mask in paper bag no. 1 and let it dry out for four days. Sunlight was not necessary. On the day 2, mask no. 2 was worn when going to duty and after returning home, was put in paper bag no. 2. The same was done for mask no. 3 and 4. N95 mask no. 1 was again used on 5th day. The exercise was repeated until all the four masks were used five times as recommended by CDC, Atlanta, USA [1,19].

All four masks were brought in a big brown bag and thrown in yellow waste bin in the area of posting after collection of all samples from masks as prescribed for the study. Each healthcare worker was doing six hours duty daily for 21 days continuously followed by one week of isolation. They were supposed to use single mask for six hours at stretch and 30 hours in total.

Out of 526 samples of N95 FFRs; Samples were taken from both external and internal surface from all N95 FFRs separately- 1st round samples (N95 masks used once only), 2nd round samples (N95 masks used twice), 3rd round samples (N95 masks used thrice), 4th round samples (N95 mask used 4 times), 5th round samples (N95 masks used 5 times) and more than 5th round samples (N95 mask used more than 5 times). Fifth round samples were considered during emergencies like tearing, misplacing or damaging of mask. Masks were disposed off after sampling. The sample collection was done just after duty of healthcare personnel got over because hourly sampling would hamper the busy working schedule of healthcare personnel during COVID-19 pandemic crisis.

The participants were asked to wear N95 FFRs for given time duration i.e., 9:00 AM to 3:00 PM. Once the specified time was over, they were asked to report the chief investigator for collection of samples from the predetermined locations. Separate swabs were used for external and internal surface of mask. After moistening the swab tips; they were swiped over twice, in a swift up and down motion at their designated area of N95 mask. Collected swabs were placed in well-labelled test-tubes, packed in Styrofoam containers and was transferred immediately to the Department of Microbiology. Swabs were immediately streaked onto blood agar, and McConkey's agar; followed by overnight incubation at 37°C. Colonies were identified based on their cultural characteristics such as size, shape, margin, edge, surface, elevation, colour of colony and on the basis of haemolysis on culture plate, and biochemical reactions such as catalase, coagulase, citrate, urease, triple sugar iron agar, indole, methyl red and Voges-Proskauer tests on manual basis. All the procedures for sample collection as well as processing was done under biosafety cabinet level 2 according to laboratory biosafety rules with the technician wearing an overall protective gown with gloves and eye cover.

STATISTICAL ANALYSIS

Collected data were entered and analysed with Microsoft excel. The variables were presented as numbers and percentages.

RESULTS

Samples were taken from both external and internal surface from all N95 FFRs separately [Table/Fig-1].

Rounds examined for culture	External surface (no.)	Internal surface (no.)
1 st	33	33
2 nd	50	50
3 rd	61	61
4 th	60	60
5 th	44	44
More than 5 th round	15	15

[Table/Fig-1]: Distribution of collection of samples from N95 FFRs.

Total 526 masks were taken for culture, out of these total masks, 362 (68.9%) masks had the growth of Gram positive bacilli. Further, the authors observed that 137 (26%) masks had the growth of micrococci. Only 8 (1.5%) masks were found to be positive for gram negative bacteria (three were *Escherichia coli* and five were *Klebsiella pneumoniae*). 11 (2.1%) masks showed the growth of *Pseudomonas*. Only 8 (1.5%) N95 mask did not had any bacterial growth [Table/Fig-2].

S. No.	Culture report	Gram Positive Bacilli (GPB) n (%)	Micrococci n (%)	Gram Negative Bacteria (GNB) n (%)	<i>Pseudomonas</i> spp. only n (%)	Clear (No bacteria) n (%)
1st Round (n=33)						
1	External	29 (87.9%)	0	0	0	04 (12.1%)
	Internal	18 (54.6%)	11 (33.3)	0	0	04 (12.1%)
2nd Round (n=50)						
2	External	40 (80%)	09 (18%)	0	01 (2%)	0
	Internal	24 (48%)	25 (50%)	0	01 (2%)	0
3rd Round (n=61)						
3	External	57 (93.4%)	02 (3.3%)	0	02 (3.3%)	0
	Internal	26 (42.6%)	35 (57.4%)	0	0	0
4th Round (n=60)						
4	External	50 (83.3%)	07 (11.7%)	01 (1.7%)	02 (3.3%)	0
	Internal	39 (65%)	21 (35%)	0	0	0
5th Round (n=44)						
5	External	31 (70.5%)	11 (25%)	01 (2.3%)	01 (2.3%)	0
	Internal	28 (63.6%)	14 (31.8%)	01 (2.3%)	01 (2.3%)	00 (0%)
More Than 5th Round (n=15)						
6	External	09 (60%)	01 (6.7%)	03 (20%)	02 (13.3%)	0
	Internal	11 (73.3%)	01 (6.7%)	02 (13.3%)	01 (6.7%)	0
7	Total (N=526)	362 (68.9%)	137 (26.0%)	08 (1.5%)	11 (2.1%)	08 (1.5%)

[Table/Fig-2]: Distribution of bacteria in relation to round of sample and surface used.

DISCUSSION

The present study evaluated bacterial load on multiple used N95 masks which shows mainly commensal bacteria. Present study

observed that the percentage of pathogenic bacteria was more after 5th round of N95 masks uses as per four mask policy.

The impact of COVID-19 pandemic in each country/region was influenced by the number of cases, rate of spread in the community, proper use of N95 FFRs, the proportion of patients needing hospitalisation and infrastructure of healthcare systems.

Respirators might also become contaminated with other pathogens acquired from patients who are co-infected with common healthcare pathogens that have prolonged environmental survival (e.g., methicillin-resistant *Staphylococcus aureus*, Vancomycin-resistant enterococci, *Clostridium difficile*, norovirus, etc.) [19].

The risks of transmission of droplet sprays or deposition of aerosolised particles on respirators with reuse depend on types of medical procedures, use of effective engineering, administrative controls etc. During bronchoscopies, sputum induction, or endotracheal intubation, there is a higher chance of respirator surface contamination. It should be emphasised that source control of patients (e.g., asking patients to wear facemasks), use of a face shield over the disposable N95 respirator, or by preventing spread of any infection is the only way to effectively control infectious pandemic [19].

In a study conducted on culture of used mouth mask done by Monalisa AC et al., it was observed that *Pseudomonas* spp. was grown in 3% of used masks while *Micrococcus* growth was found only in 1% of masks. *Escherichia coli* (gram negative bacilli) and *Klebsiella* spp. (gram negative coccobacilli) were found in 54% and 5% of masks, respectively. *Staphylococcus aureus* (gram positive cocci) was found in 25% of samples [20].

In study of Luksamijarulkul P et al., a total of 230 used masks were collected from 214 personnel to assess the bacterial and fungal contamination [21]. Results revealed that isolated bacteria contaminated on inside and outside areas of the used masks were *Staphylococcus* spp. (34% and 41%, respectively) and *Pseudomonas* spp. (34% and 38%, respectively). Thus, results of Gram positive bacilli growth are almost in concordance with the result of present study. The growth of *Pseudomonas* spp. was found in smaller number of used masks (2.1%) in present study which was contradictory to the Luksamijarulkul P et al., study.

In study by Mills D et al., it was concluded that "FFR decontamination and reuse using UVGI can be effective. Implementation of a UVGI method will require careful consideration of FFR model, material type, and design" [22].

In a study done by Chughtai AA et al., as done to determine the areas of masks likely to contain maximum viral particles. Overall virus positivity rate was 10.1% (15/148). Commonly isolated viruses from masks samples were adenovirus (n=7), bocavirus (n=2), respiratory syncytial virus (n=2) and influenza virus (n=2). Virus positivity was significantly higher in masks samples worn for >6 hour (14.1%, 14/99 versus 1.2%, 1/49, Odds Ratio (OR) 7.9, 95% Confidence Interval (CI) 1.01-61.99) and in samples used by participants who examined >25 patients per day (16.9%, 12/71 versus 3.9%, 3/77, OR 5.02, 95% CI 1.35-18.60) [23]. The present study correlates well with present study but viral parameters were used in this study while in present study bacterial contamination was advocated.

Two studies (Vuma CD et al., Bergman MS et al.) reported that 7% to 8% of N95s failed fitting after two uses and >20% failed after five fittings [24,25].

Park AM et al., studied low bacterial count in female that could be associated with a more intensive facial skincare by females than by males [26]. Also, high *Cladosporium* fungi were found in their study. Bacterial colony count was variable (1-1600 per plate) and fungal colony was 1-22 colonies per plate. Bacterial load was high on the face side mask as compared to fungal load which was more on outer side. Duration of usage had no effect on bacterial colonisation but fungal colonisation was more common in two days user.

Continued importance of reinforcing good hand hygiene after Personal Protective Equipment (PPE) removal is required for preventing the spread of infection.

Prevention can be done by breaking chain of transmission via aerosol droplets and surface contact which can be achieved by social distancing, staying indoors, washing hands regularly with soap atleast 20 seconds or using alcohol-based hand sanitisers and most preferably by using N95 FFRs. Due to short supply of these, alternative strategies were also applicable for judicious use of these N95 respirators.

Limitation(s)

Only specimens of the healthcare workers were sampled but not from patients. The persons collecting the samples must be blinded for the duration of use of N95 FFRs. The present study was a single centre study, preferably must be multicentric.

CONCLUSION(S)

Health authorities should consider global N95 FFRs shortage and provide recommendations which are feasible for reuse of N95 respirators. Regulatory agencies must implement strategies for decontamination and/or reusing procedures. The reuse of N95 respirators have become the last resort, crucial to maintaining the healthcare worker protected during the response to COVID-19 pandemic and other respiratory virus pandemic. Therefore, four masks policy can be easily applied in OPD settings for extended use as well as reuse. It will further also help in reducing the economic burden on the government.

REFERENCES

- [1] All India Institute of Medical Sciences New Delhi- Guidelines Issued on "Standard Operating Procedure (SOP) for extended use of N-95 mask for personnel safety of Healthcare Workers (HCW) at AIIMS."; Dated 8/April /2020. https://www.aiims.edu/images/pdf/notice/SOP_N95_09_04_20.pdf.
- [2] "COVID-19". Oxford English Dictionary (Online ed.). Oxford University Press. April 2020.
- [3] "ECRI:" Clinical Evidence Assessment Safety of Extended Use and Reuse of N95 Respirators. (March 2020). https://www.elsevier.com/_data/assets/pdf_file/0006/997863/COVID-ECRI-N95-Respirators_2020-03.pdf.
- [4] CDC: "Guidelines for preventing the transmission of Mycobacterium tuberculosis in healthcare facilities." [Online] Available at: <https://www.cdc.gov/mmwr/pdf/rr/r4313.pdf> pdf icon, 1994). <https://www.cdc.gov/mmwr/preview/mmwrhtml/rr5417a1.htm>.
- [5] CDC: "Questions and Answers Regarding Respiratory Protection for Preventing 2009 H1N1 Influenza Among Healthcare Personnel" [Online] Available at https://www.cdc.gov/h1n1flu/guidelines_infection_control_qa.htm, 2010). https://www.cdc.gov/h1n1flu/guidelines_infection_control_qa.htm.
- [6] Rebmann T, Alexander S, Cain T, Citarella B, Cloughessy M, Coll B. APIC position paper: extending the use and/or reusing respiratory protection in healthcare settings during disasters. [Online] Available at: http://www.apic.org/Resource/TinyMceFileManager/Advocacy-PDFs/APIC_Position_Ext_the_Use_and_or_Reus_Resp_Prot_in_Hlthcare_Settings1209L.pdf pdf iconexternal icon, 2009). http://www.apic.org/Resource/TinyMceFileManager/Advocacy-PDFs/APIC_Position_Ext_the_Use_and_or_Reus_Resp_Prot_in_Hlthcare_Settings1209L.pdf.
- [7] Casanova L, Rutala WA, Weber DJ, Sobsey MD. Coronavirus survival on healthcare personal protective equipment. *Infect Control Hosp Epidemiol*. 2010;31(5):560-61.
- [8] Coulliette A, Perry K, Edwards J, Noble-Wang J. Persistence of the 2009 Pandemic Influenza A (H1N1) Virus on N95 Respirators. *App Environ Microbiol*. 2013;79(7):2148-55.
- [9] Fisher EM, Shaffer RE. Survival of bacteriophage MS2 on filtering facepiece respirator coupons. *Appl Biosaf: J Am Biol Saf Assoc*. 2010;15(2):71.
- [10] Lopez GU, Gerba CP, Tamimi AH, Kitajima M, Maxwell SL, Rose JB. Transfer efficiency of bacteria and viruses from porous and nonporous fomites to fingers under different relative humidity conditions. *Appl Environ Microbiol*. 2013;79(18):5728-34.
- [11] Fisher EM, Ylitalo CM, Stepanova N, Shaffer RE. Assessing filtering facepiece respirator contamination during patient care in flu season: Experimental and modeling approaches. in *isrp-sixteenth international conference: A global view on respiratory protection*. Boston, 2012. <https://www.isrp.com/boston-2012-papers/19-edward-m-fisher-caroline-m-ylitalo-narina-stepanova-ronald-e-shaffer/file>.
- [12] Rusin, P, Maxwell S, Gerba C. Comparative surface-to-hand and fingertip-to-mouth transfer efficiency of gram-positive bacteria, gram-negative bacteria, and phage. *J Appl Microbiol*. 2002;93(4):585-92.
- [13] Fisher EM, Richardson AW, Harpest SD, Hofacre KC, Shaffer RE. Reaerosolization of MS2 bacteriophage from an N95 filtering facepiece respirator by simulated coughing. *Ann Occup Hyg*. 2012;56(3):315-25.

[14] Birkner JS, Fung D, Hinds WC, Kennedy NJ. Particle release from respirators, part I: determination of the effect of particle size, drop height, and load. *J Occup Environ Hyg.* 2011;8(1):01-09.

[15] Kennedy NJ, Hinds WC. Release of simulated anthrax particles from disposable respirators. *J Occup Environ Hyg.* 2004;1(1):07-10.

[16] Willeke K, Qian Y. Tuberculosis control through respirator wear: Performance of National Institute for Occupational Safety and Health-regulated respirators. *Am J Infect Control.* 1998;26(2):139-42.

[17] Chen X, Ran L, Liu Q, Hu Q, Du X, Tan X. Hand hygiene, mask-wearing behaviors and its associated factors during the COVID-19 epidemic: A cross-sectional study among primary school students in Wuhan, China. *Int J Environ Res Public Health.* 2020;17(8):2893. Doi: 10.3390/ijerph17082893.

[18] Ma QX, Shan H, Zhang HL, Li GM, Yang RM, Chen JM. Potential utilities of mask-wearing and instant hand hygiene for fighting SARS-CoV-2. *J Med Virol.* 2020;92(9):1567-71. Doi: 10.1002/jmv.25805.

[19] CDC: "Recommended Guidance for Extended Use and Limited Reuse of N95 Filtering Facepiece Respirators in Healthcare Settings." [Online] Available at: <https://www.cdc.gov/coronavirus/2019-ncov/index.html>. <https://vamedicine.ucsf.edu/sites/vamedicine.ucsf.edu/files/inline-files/Recommended%20Guidance%20for%20Extended%20Use%20and%20Limited%20Reuse%20of%20N95%20Filtering%20Facepiece%20Respirators%20in%20Healthcare%20Settings.pdf>.

[20] Monalisa AC, Aruna CN, Padma KB, Manjunath K, Hemavathy E, Varsha D. Microbial contamination of the mouth masks used by post-graduate students in a private dental institution: An In-Vitro study. *IOSR J Dent Med Sci.* 2017;16:61-67.

[21] Luksamijarulkul P, Aiempredit N, Vatanasomboon P. Microbial contamination on used surgical masks among hospital personnel and microbial air quality in their working wards: A hospital in Bangkok. *Oman Med J.* 2014; 29(5): 346-50. Doi: 10.5001/omj.2014.92.

[22] Mills D, Harnish DA, Lawrence C, Sandoval-Powers M, Heimbuch BK. Ultraviolet germicidal irradiation of influenza-contaminated N95 filtering facepiece respirators. *Am J Infect Control.* 2018;46(7):e49-e55. Doi: 10.1016/j.ajic.2018.02.018.

[23] Chughtai AA, Stelzer-Braid S, Rawlinson W, Pontivivo G, Wang Q, Pan Y, et al. Contamination by respiratory viruses on outer surface of medical masks used by hospital healthcare workers. *BMC Infect Dis.* 2019;19:491.

[24] Vuma CD, Manganyi J, Wilson K, Rees D. The effect on fit of multiple consecutive donning and doffing of N95 filtering facepiece respirators. *Ann Work Expo Health.* 2019;63(8):930-36.

[25] Bergman MS, Viscusi DJ, Zhuang Z, Palmiero AJ, Powell JB, Shaffer RE. Impact of multiple consecutive donnings on filtering facepiece respirator fit. *Am J Infect Control.* 2012;40(4):375-80.

[26] Park AM, Khadka S, Sato F, Omura S, Fujita M, Hashiwaki K, et al. Bacterial and fungal isolation from face masks under the COVID-19 pandemic. *Sci Rep.* 2022;12(1):11361. Doi: 10.1038/s41598-022-15409-x. PMID: 35851044; PMCID: PMC9293923.

PARTICULARS OF CONTRIBUTORS:

1. Principal Specialist, Department of Ophthalmology, Government RDBP Jaipuria Hospital, Jaipur, Rajasthan, India.
2. Associate Professor, Department of Obstetrics and Gynaecology, Government RDBP Jaipuria Hospital and RUHS College of Medical Sciences, Jaipur, Rajasthan, India.
3. Professor and Head, Department of Surgery and Nodal Officer COVID-19, Government RDBP Jaipuria Hospital and RUHS College of Medical Sciences, Jaipur, Rajasthan, India.
4. Assistant Professor, Department of Obstetrics and Gynaecology, Government RDBP Jaipuria Hospital and RUHS College of Medical Sciences, Jaipur, Rajasthan, India.
5. Medical Officer, Department of Pathology, Government RDBP Jaipuria Hospital, Jaipur, Rajasthan, India.
6. Professor and Head, Department of Microbiology, RUHS College of Medical Sciences, Jaipur, Rajasthan, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:

Dr. Gaurav Dalela,
 Professor and Head, Department of Microbiology, RUHS College of Medical Sciences,
 Jaipur-302033, Rajasthan, India.
 E-mail: gauravdalela29@gmail.com

PLAGIARISM CHECKING METHODS: [Jain H et al.]

- Plagiarism X-checker: Sep 27, 2022
- Manual Googling: Mar 14, 2023
- iThenticate Software: Apr 05, 2023 (15%)

ETYMOLOGY: Author Origin

EMENDATIONS: 6

AUTHOR DECLARATION:

- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? Yes
- For any images presented appropriate consent has been obtained from the subjects. NA

Date of Submission: **Sep 21, 2022**
 Date of Peer Review: **Nov 23, 2022**
 Date of Acceptance: **Apr 08, 2023**
 Date of Publishing: **Jul 01, 2023**