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Indoor air microbial quality in medical emergency of an Algerian hospital

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ABSTRACT

Introduction: In this work, we target the analysis and the characterization of bioaerosols species present in medical emergency of north Algerian hospital, were we consider in four operating rooms, two preoperative and resuscitation rooms.

Materials and methods: Passive technique was applied for the collection of bacterial and fungal samples in indoor air, for three days from 16 to 18 January 2018. Two techniques were then chosen for the bacterial identification, the Analytical Profile Index (API) system and Matrix Assisted Laser Desorption/ Ionization Time of Flight Mass Spectrometry (MALDI-TOF MS).

Results: It has been found that fungal contamination was highest in neurosurgery block, 103 CFU/m^3, whereas the highest bacterial contamination, 2645 CFU/m^3, was noted in postoperative room. The most predominant identified bacteria were Gram-positive cocci.

Conclusion: The high contamination in bioaerosols and the types of bacteria identified in the premises studied increase the risk of contracting a nosocomial infection, hence the importance of daily disinfection and sterilization of hospitals area in order to reduce bioaerosols dangerous effects on human health.

Introduction

In recent years, a big attention has been paid to analysis of bioaerosols in both occupational and indoor environment. This generally consists of studying airborne particles that originate from biological sources and the evaluation of their important impact on human health, as respiratory infections, allergic reactions or more complicated illnesses [1-4]. Such negative effects are generally dependent of type of microorganism's presents, their concentrations and duration of exposure [5, 6]. Many studies have been carried out on the analysis of Bioaerosols (BAs) in professional environments [7-15], such

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as schools and universities [16, 17], medical environments [18-28], homes [29-31], sports Halls [32] and hammams [33]. Moreover, many reported studies has focused on controlling internal pollution in hospital environments [34-40], which are highly sensitive since they contain different varieties of BAs species, particularly bacteria and fungi, causing multiple diseases until dangerous cancer [26, 35, 41]. The predominant identified bacteria include gram-positive strains such as Staphylococcus and Micrococcus. As for fungi, the most commonly identified species are Aspergillus and Penicillium [42, 43]. Indeed, the risk of getting an infection is even higher in healthcare departments where patients are more susceptible, or in operating rooms because of tissue exposure to air. The risk is even greater when it comes to emergency medical operating rooms, where immediate surgical interventions are typically carried out, especially in cases of severe trauma. It has been demonstrated that periprosthetic infection rates correlate with the number of airborne bacteria within the wound [44]. Inhalation of Mycobacterium tuberculosis can reach the lungs and cause tuberculosis [45].

The importance of studying BAs concentrations in a medical environment is explained by the sensitivity of this medium, and the continuous evolution of several physical, chemical and biological parameters in the internal environment of healthcare facilities. These parameters can generate direct and cross-pollution [46]. Several factors can affect the quality and concentrations of these BAs species in the indoor medical environment, such as season (temperature, humidity, air exchange rate, air movement), weather conditions, ventilation system, building materials, intrusion of moisture, outdoor microbial load, number of occupants, visitors, human activities, medical activities, cleaning frequencies and cleaning procedures [11, 20, 47-49]. Pathogens with high resistance such as methicillin-resistant Staphylococcus aureus (MRSA) may spread via the aerial route, leading to an increase in hospital-acquired infections and the spread of antibiotic resistant genes [50].

To the best of our knowledge, only a few studies have been reported on the quality of indoor air in Algeria [51-54] especially on the biological fraction [9, 25, 27, 46, 55], although World Health Organization (WHO) places great importance on monitoring BAs in indoor air [49].

In an attempt to emphasize the importance and obligation of monitoring BAs in indoor air in hospital and to sensitize health professionals to reduce their rates, especially in operating rooms, we report in this study the quantification and identification of BAs in the medical emergencies of a north Algerian hospital, considering various specific locations. The Analytical Profile Index (API) system and Matrix Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry (MALDI-TOF MS) were extensively employed.

Materials and methods

Study area

The study was carried out in the medical emergency building in north of Algeria. Built in 1933, with a total area of 35 hectares and a total current capacity of 1613 beds, formerly psychiatric facility.

The sampling was realized at three operating rooms, post-operative, resuscitation rooms and septic block. In each operating room, there is a central air conditioner, an air extractor that works once or twice a week and a sterile block that is started every 48 h with a sterilization time of 4 h. Additionally the sterile block is activated after each encounter with immunosuppressed patient. Table 1 summarizes the specialty of operating rooms and the average of surgery by day with the occupancy during surgery. For the septic room, the sterile block is started after each patient. The frequency of cleaning surfaces in operating rooms is after each surgery. It should be noted that sometimes the sterile block and ventilation does not work, the reason for what the cleaning frequencies are increased in these cases.

Table 1. Specialty of operating rooms and the average of surgery by day with the occupancy during surgery

Operating rooms	Average of surgery by day	Average occupancy during surgery
General surgery block (L1)	4	8
Neurosurgery block (L2)	2	4
Traumatology block (L3)	7	8

Sampling procedure

The measurement of bacteria and fungi at different operating rooms were made by passive air sampling technique over a period of 3 three days, from 16 to 18, January 2018. One sample per day of bacteria and fungi was carried out at 10 a.m, using Petri dishes (9 cm diameter), containing culture media left open to the air according to the 1/1/1 scheme (for 1 h, 1m from the floor, at least 1 m away from walls or any obstacle) according to index of microbial air contamination [56]. We have used nutrient agar and Sabouraud agar for bacteria and fungi respectively. After exposure, the Petri dishes were closed by the parafilm, stored and transported to the laboratory for incubation.

Bacteria and fungi were incubated at 37 °C for 3 days and at 25 °C for 5 days respectively, then a manual counting under a light source of colony pushed for both bacteria and fungi was realized. The determination of CFU/m^3 is made by the Eq. 1 [11]. The identification of bacteria was realized by two methods: Analytical Profile Index (API) system and Matrix Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry (MALDI-TOF MS).

$$N=5a\times10^4\ (bt)^{-1} \quad (1)$$

Where;

N = Microbial concentration of indoor air (CFU/m^3);

a = Number of colonies per Petri dish;

b = Surface area of the dish (cm^2);

t = Exposure time (min)

Laboratory method for bacterial identification

After incubation, colonies with different aspects and colors were observed. Each colony is isolated on cooked blood agar until complete purification, then gram stain, oxidase and catalase are performed on each pure colony. The majority of bacterial colonies were characterized as Gram-positive cocci, being cultivated on Chapman medium followed by identification by API system and by MALDI-TOF MS.

Bacterial identification by analytical profile index (API)

After sample preparation, colonies were emulsified into the API medium to achieve a homogeneous bacterial suspension of a 0.5 McFarland standard. A sterile syringe was used to distribute the bacterial suspension into the tubes and the cups are filled with specific reagents. The incubation of strips was realized at 37°C for 24 h. The strips were read and the

7-digit numerical profile was obtained. The interpretation of results was performed with the analytical profile index by looking up the numerical profile in the list of profiles.

Bacterial identification by MALDI-TOF MS

The MALDI-TOF MS Microflex LT mass spectrometer (Bruker Daltonik, Germany) with FlexControl (version 3.4) and biotyper, was also applied as second technique for bacterial identification, using the direct transfer method. The extended method was also used as second plan when no peaks were found with the direct method, so we realized one or more subculture of some colonies for the crystallization of their proteins.

The direct method consist to smear biological material, fresh single colony from nutrient agar on the steel target plate and overlay the material with 1 µL of matrix solution [4-hydroxy-a-Cyanocinnamic Acid (HCCA)], within 1 h and dry at room temperature then the target was transferred to the MALDI-TOF MS for analysis.

For extended method, fresh colony was smeared on the target overlaid with1 µL of 70% formic acid dried at room temperature, even its dry 1 µL of HCCA matrix is added, a second air-dried is necessary before introducing the sample for analysis by MALDI-TOF MS. Both methods are realized according to the manufacturer's instructions. Data acquisition is done in linear mode detector set 2558 v monitor 2555 v, with mass range [1986-20137] Da. UV is the source of Laser with a frequency of 60 hz and the number of shots is 40; high voltage and positive polarity.

The Bacterial Test Standard (BTS) is Escherichia coli ATCC 25922 THL in dehydrated form. The interpretation of the identification score is based on the scale recommended by the manufacturer, highly probable species identification [2.300-3.000] ; secure genus identification, probable species identification [2.000-2.299] ; probable genus identification [1.700-1.999] ; not reliable identification [0.000-1.699].

Statistical analysis

Data were analyzed using Statistical Package for Social Sciences (SPSS) version 20. One way ANOVA test was conducted to assess the statistical distribution and to obtain the min/max values, the mean and the mean standard error of bacterial and fungal concentration recorded in the seventh locations of the investigated hospital during three days. Also, the likelihood of statistically significant differences (T student test) between the concentrations of bacteria and fungi measured at different sampling locations and the linearity was determined between the concentrations of bacteria and fungi results.

Results and discussion

Concentrations of Bacteria and fungi BAs in the indoor air of a medical emergency setting, estimated by the passive air sampling method were found ranged between [191-2645] and [6-147] CFU/m^3 respectively.

The highest bacterial concentration noted in postoperative 2 reached 2645 CFU/m^3, while the lowest was 191 CFU/m^3 considered in the traumatology block (Table 2). The concentrations of bacteria measured in all locations were significantly different to each other (P range from 0.002 to 0.045).

The highest Fungal concentration, amounting to 147 CFU/m^3, was detected in resuscitation Room, while the lowest measuring 6 CFU/m^3, was found in poste-operative 1, (Table 2). The concentrations of fungi measured in location 2, 4, 5, 6 and 7 record not significant difference to each other (P=0.054 to 0.172). However, the general surgery block and the traumatology block exhibited a significant difference with concentration of 64±5 CFU/m^3 (P=0.005) and 24±5 CFU/m^3 (P=0.035) respectively.

Table 2. Statistical distribution of airborne bacteria and fungi (CFU/m^3) according to the sampling day (D1 to D3) and the location (L1 to L7)

Distribution			Valid N	Min	Max	Mean	Mean Standard Error
General		Bacteria	21	191	2645	796	138
		Fungal	21	6	147	55	7
Per Day	D1	Bacteria	7	338	1852	865	214
		Fungal	7	15	147	71	18
	D2	Bacteria	7	367	1911	705	204
		Fungal	7	29	103	57	9
	D3	Bacteria	7	191	2645	819	317
		Fungal	7	6	59	39	7
Per Location	L1	Bacteria	3	514	896	646	125
		Fungal	3	59	73	64	5
	L2	Bacteria	3	338	485	397	45
		Fungal	3	15	103	54	26
	L3	Bacteria	3	191	632	460	136
		Fungal	3	15	29	24	5
	L4	Bacteria	3	647	1411	1009	221
		Fungal	3	6	73	46	20
	L5	Bacteria	3	1852	2645	2136	255
		Fungal	3	29	73	54	13
	L6	Bacteria	3	338	544	447	59
		Fungal	3	44	147	78	34
	L7	Bacteria	3	441	529	485	25
		Fungal	3	44	103	69	18

L1: general surgery block; L2: neurosurgery block; L3: traumatology block; L4: post-operative 1; L5: post-operative 2; L6: resuscitation room; L7: septic block.

D1: 16.01.18; D2: 17.01.18; D3: 18.01.18.

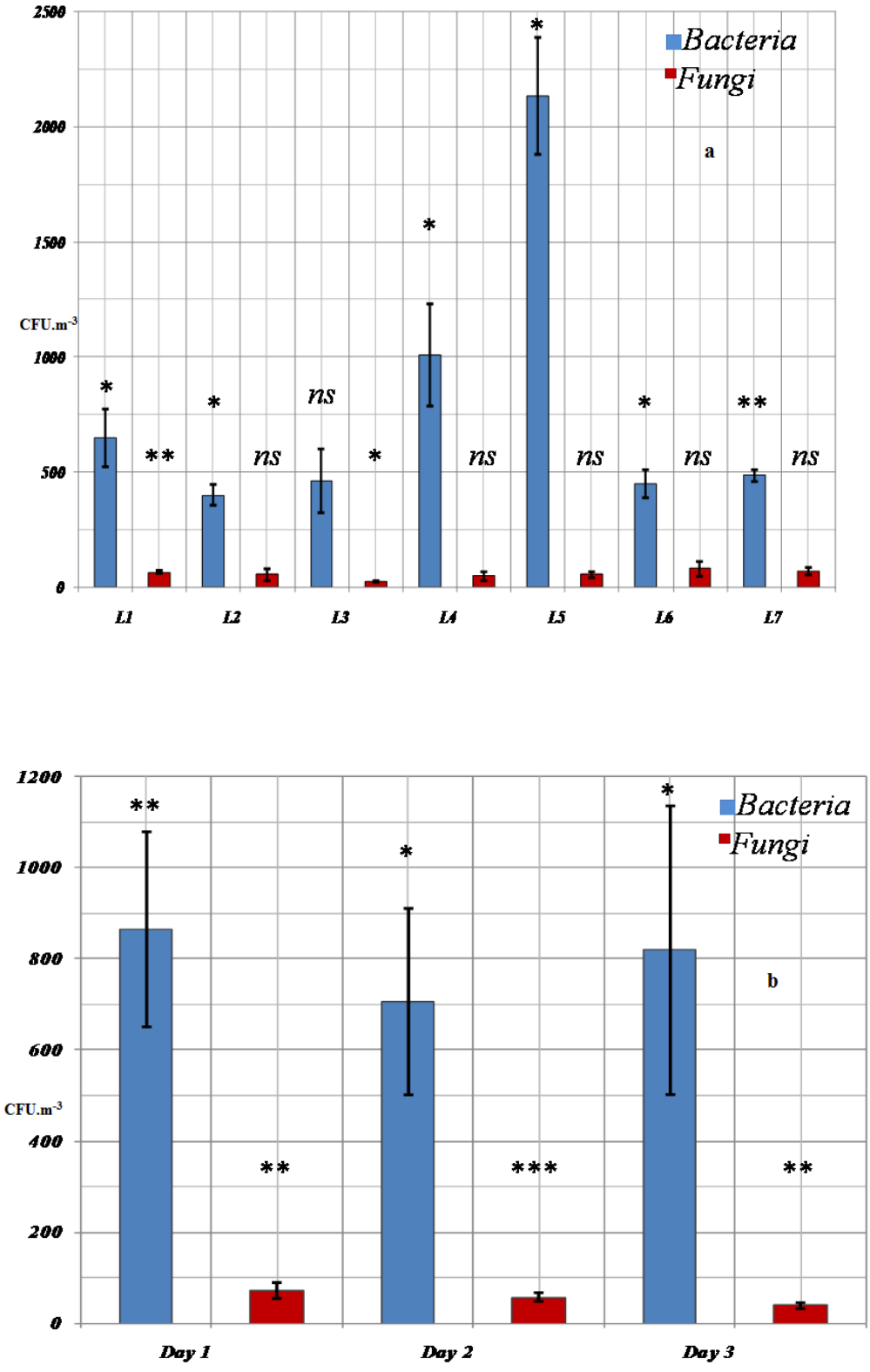

Fig. 1. The mean distribution of airborne bacterial and fungal concentration (CFU/m^3) in the medical emergencies. (a) according the 7 locations (b) according the three days

ns: not significant difference; * Significant difference; ** Highly Significant difference; *** Very Highly Significant difference. L1: general surgery block; L2: neurosurgery block; L3: traumatology block; L4: post-operative 1; L5: post-operative 2; L6: resuscitation room; L7: septic block. D1: 16.01.18; D2: 17.01.18; D3: 18.01.18.

Internationally, concentration limits for bioaerosols are imposed, and must be respected. Indeed, the United Kingdom limit these values at 35 CFU/m^3 for an empty operating rooms and it should not exceed 180 CFU/m^3 in activity, for an average of 5 min period. The American conference of governmental industrial hygienists (ACGIH) requires a limit of 100 CFU/m^3 [58]. World Health Organization limited at 500 CFU/m^3 for bacteria and fungi [49].

On the basis of the recorded values, we deduce that the fungal concentration were under the limits recommended by ACGIH and WHO [49, 58], except L2 in day 2, L6 in day 1 and L7 in day 1, where the values exceed limits. In the other hand, number of airborne bacterial at different sampling of the three days exceed the limits, except L2, L3 in day 3, L6 and L7 in days 1 and 2, where the values were under the limit of WHO. These obtained results were generally in agreement with values found in the study of microbial quality of indoor air in hospital at Pakistan, reporting that bacterial load in operating rooms were [60-466.7] CFU/m^3 while fungal load were [0-266.7] CFU/m^3. In the emergency services, the bacterial concentration was equal to [280-2280] CFU/m^3 and fungal concentration were equal to [19.2-384.6] CFU/m^3 [20]. Also, the study of microbial air quality at different hospital sites in Portugal reported values moderately similar to ours in emergency service ([240-736] CFU/m^3 for bacteria and [27-933] CFU/m^3 for fungi), but in operating rooms, their values were low comparing to the reported values in this work ([12-170] CFU/m^3 for bacterial levels and fungal levels were below 1 CFU/m^3) [48]. Another study conducted in a hospital in Iran reported minimum to maximum values of [119-835] for bacteria and [28-49] for fungi [57]. The values reported in the bioaerosols evaluation in five educational hospitals in Iran were very low compared to the values found in this work [59]. Additionally, these values were significantly lower than those reported in the study [39]. The high bioaerosols BAs concentrations in medical emergency of considered hospital can be explained by the nature of chosen sites which were operating rooms of medical emergency, so they were all time occupied by patient infected with different diseases, under responsibility of a medical service in continuous activities. Indeed, it has been reported that the variation of BAs concentrations depends on the occupancy in hospital rooms and the human activity like coughing, sneezing, walking, washing and talking [11, 48, 49, 60, 61]. Also, it has been found that the disinfection and sterilization directly influences the concentration of bacteria and fungi in the indoor air and the difference is significant before and after cleaning [20, 35]. In the case of considered operating rooms, as shown in Table 1, the occupancy varied between 2 to 8 persons and generally, in reason of urgency, the frequency of cleaning and sterilization was not always respected.

The postoperative 1 and 2 are characterized by too high contaminations. This result may be relative in postoperative 2 to the number of beds since there were an average of ten beds. However, there was only one bed in postoperative 1, which is generally reserved to the sensitive patients, but we mustn't forget to take into consideration the number of people from medical service practicing in this room. Indeed, this room was relatively isolated it is important to note that the room is busy all the time which increases the number of BAs in the air. So we can deduce that the postoperative 1, was relatively isolated in the great hall, but had a cross contamination under the effect of opened door and people passage.

The identification of isolated bacteria in the different medical emergency departments was carried out by two techniques API system and

MALDI-TOF MS. Both techniques give similar results. The identification score for MALDI-TOF MS varies between 1.722 and 2.216, which gives genus identification for the values included in the interval [1.700-1.999] and species identification for the values included in the interval [2.000-2.299]. Those results are presented in Table 3.

Table 3. Identification of airborne bacteria in medical emergency by MALDI-TOF MS and API

Sampling site	MALDI-TOF MS identification (Identification score)	API system identification
General surgery block	*Staphylococcus xylosus* DSM 6179 DSM (1.796) *Kocuria marina* DSM 16420T DSM (2.005) *Kocuria rosea* IMET 11363T HKJ (2.216)	*Staphylococcus xylosus* *Staphylococcus warneri* *Kocuria marina sp* *Staphylococcus lentus* *Micrococcus ssp*
Neurosurgery block	*Kocuria rosea* DSM 11630 DSM (1.9)	*Kocuria varians* *Staphylococcus cohnii cohnii*
Traumatology block	*Staphylococcus hominis* 18 ESL (1.933) *Micrococcus luteus* 59 PIM (1.707) *Arthrobacter oxydans* IMET 10684T HKJ (1.952)	*Staphylococcus sciuri* *Micrococcus spp* *Kocuria rosea*
Post-operative 1	*Staphylococcus hominis* 18 ESL (2.055) *Kocuria rosea* IMET 11363T HKJ (1.84)	*Staphylococcus hominis* *Staphylococcus cohnii cohnii* *Micrococcus ssp* *Kocuria rosea* *Cellulomonas sp* *Cellulomonas microbacterium*
Post-operative 2	*Staphylococcus xylosus* DSM 20266T DSM (1.722) *Staphylococcus haemolyticus* 10024 CHB (1.909) *Micrococcus luteus* IMET 11249 HKJ (1.733)	*Staphylococcus cohnii cohnii* *Staphylococcus hominis* *Micrococcus ssp* *Staphylococcus sciuri*
Resuscitation room	*Kocuria rosea* B331 UFL (1.933)	*Staphylococcus cohnii cohnii* *Kocuria varians* *Kocuria rosea*
Septic block	*Staphylococcus haemolyticus* DSM 20264 DSM (2.107)	*Staphylococcus auricularis* *Staphylococcus cohnii cohnii* *Micrococcus ssp*

We identified staphylococci including Staphylococcus xylosus, Staphylococcus warneri, Staphylococcus lentus, Staphylococcus cohnii cohnii, Staphylococcus sciuri, Staphylococcus hominis, Staphylococcus auricularis, and Staphylococcus haemolyticus. Micrococcus including Micrococcus luteus, Kocuria including Kocuria marina sp, Kocuria rosea, and Kocuria varians, an Arthrobacter oxydans and Cellulomonas sp cellulomonas microbacterium. All the bacteria identified are Gram-positive cocci. These results are in agreement with the literature [20, 43, 48, 62]; indeed, the predominant isolated genera were gram positive (97%) [20], and 88% of gram positive [48]. In this study we did not identify any gram negative bacteria. This can be explained by the use of the passive sampling technique, so in the sampling area there were no gram negative bacteria identified.

The presence of these bacteria in the air comes from several origins, from the environment (including building, soil, alkaline waste water, dust, water, and air) and from human and animals. These bacteria have a harmful effect on the health of the human; they can cause irritation, meningitis, prosthetic join infections, skin infection and food poisoning [20, 33, 48, 62].

Conclusion

This study emphasizes the importance of analyzing and monitoring the microbiological quality of indoor air in hospitals. Microbiological agents suspended in the air can be pathogenic to humans; causing infections and increasing the risk of nosocomial infections. The risk is even greater in operating rooms where bioaerosols come into direct contact with the patient's cellular tissue, particularly when dealing with immunosuppressed patients.

This contribution represents one of the first studies conducted in Algeria, especially within a hospital environment. The obtained results reveal that BAs concentrations in operating rooms and other areas exceed the thresholds set by WHO and other international standards. Gram-positive cocci were identified as the dominant bacteria in the indoor air of emergency medical in considered hospital.

It is recommended to consider the points that favor the persistence in the air of these BAs, in order to reduce their concentrations in the indoor air. This involves the installation and maintenance of air handling and ventilation systems, disinfection and sterilization, humidity control, specific coating of walls, floors, ceilings and worktop laboratory in the biological room, implantation of good hygienic practices, raising awareness among hospital practitioners and the creation of national regulations to limit of BAs load with periodic monitoring in hospitals.

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Competing interests

The authors declare that there are no competing interests.

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Ethical considerations

Ethical issues (Including plagiarism, Informed Consent, misconduct, data fabrication and/ or falsification, double publication and/ or submission, redundancy, etc.) have been completely observed by the authors.

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