Original Research Article

BACTERIOLOGICAL ASSESSMENT OF CRITICAL AREAS IN THREE SELECTED UNITS IN JODHPUR DENTAL COLLEGE GENERAL HOSPITAL, JODHPUR RAJASTHAN, INDIA

Abstract

Introduction: the hospital environment plays a crucial role in the chain of infection spread. Thus there is need to attack the chain of infection at it weakest link as the most effective way in combating and/or preventing nosocomial infections. The study aims to assess the common aerobic pathogenic bacteria in the different departments of Jodhpur National University Dental Hospital and to establish the best disfectant as well as disinfection procedure.

Methods and Materials: A cross sectional descriptive study was conducted. Air and surface samples were taken before and after dental procedures from all the three departments within durations of 14 days. Different antiseptics were used so as to compare their effectiveness. Swabs taken from different places were streaked on blood agar plates and incubated at 37^{o}C under aerobic conditions for 24 hours. After incubation, isolates obtained were appropriately identified. After aerobic incubation of the settle plates at 37^{0}C for 24hours, the colonies on each plate were counted and recorded as the number of bacteria carrying particles settling over the area of the plate in a given period of time. The level of bacterial contamination of air is usually expressed as the number of bacteria carrying particles per cubic millimeter.

Results: A total of 274 surface samples and 97 air samples were collected. Bacteria were isolated in all air samples while only 255 surface samples had growth. The predominant species in all services was Bacillus spp, followed by coagulase negative Staphylococci, *Micrococcus luteus,*

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aerobic spore formers and least was *Pseudomonas aeruginosa.* The bacteria isolated in the air were similar to those isolated from surfaces.

Conclusion: In conclusion, lack of a universal procedure for surveillance of nosocomial infection, presence of pathogenic bacteria, poor hand hygiene and heavy contamination of some important surfaces are the most important problems in our hospitals.

Keywords: Swab, Bacillus spp, isolates, culture, sensitivity, infection, growth

Introduction

Attacking the chain of infection at its weakest link is the most effective procedure in combating and/or preventing nosocomial infections .[3] The environment plays an important role in the chain of acquiring an infection. Although surfaces which are microbiologically contaminated can serve as reservoirs of pathogens, they are not associated with infection transmission to either staff or patients directly .[10] Direct hand contact with surfaces is largely the route of transmission of microorganisms from environmental surfaces to patients. Even though proper hand hygiene is essential to minimize the impact of this transfer, appropriate cleaning and disinfection of environmental surfaces is necessary to reduce their potential risk to the incidence of healthcare-associated infections .[11]

There is need for proper ventilation in the hospital and other healthcare facilities for patients' comfort as well as control of hazardous emissions .[4,15] In addition to fellow patients, patients may serve as source of infectious microorganisms to staff and hospital visitors, thus the concern for biological quality of air in the hospital .[16] Infected patients are the main source airborne pathogens inside the hospital .[9] When an infected person transmits pathogenic microorganisms to a susceptible individual through the air, airborne transmission is said to occur.[2] The production of aerosol droplets by sneezing or coughing makes a pathogen airborne by their subsequent loss of water, thereby allowing them to float in the air which spread over considerable distances and for a while thus contaminating surfaces they come in contact with.

The introduction of pathogenic microorganisms into the body also initiates a nosocomial infection as a result of hospitalization and/or certain medical procedures which are mainly meant to cure diseases .[2] There is need for hospital management to ensure that water used in their

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healthcare facilities is safe to drastically reduce any potential risk of infection to people receiving services within such hospital environment.

The healthcare environment is a contributing factor to healthcare associated infections as such plays a crucial role in infection transmission. The personnel, the patients or the inanimate environment may be the source of infection due to contamination in any healthcare facility. Microbial contamination of hospital environment, especially in an operating theatre and other specialized units had continued to increase prevalence of nosocomial infections. In the study carried out by Maki and his colleagues [14] in trying to assess the relationship between organism on environmental surface and nosocomial infection, they virtually ruled out the environment as a significant vector for nosocomial infections. Inanimate surfaces have often been described as the source for outbreaks of nosocomial infections.

This study aims to assess the common aerobic pathogenic bacteria in three different departments (Periodontics & Implantology, Conservative & Endodontics and Oral surgery) of Jodhpur National University Dental Hospital and to establish the best disfectant as well as disinfection procedure.

Objectives of the Study

1. Microbial Monitoring of surfaces and Air
2. To analyze the pattern of antibiotic-sensitivity of potential pathogens isolated.
3. To compare different sterilizing agents so as to understand the most effective.

Laboratory Materials/Equipment

Petri dishes, sheep blood, nutrient agar, MacConkey agar, sterile swab sticks, distilled water, autoclave, refrigerator, incubator, nutrient broth , antibiotic sensitivity disc, test tubes, Gram stains, microscope, wire loop,

Sample Size Determination

The sample size was calculated using the equation below .[13,18]

$$n = \frac{Z^2 Pq}{d^2}$$

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Z=1.96 (95%)

P= previous study prevalence

P= 50% = 0.5

q= 1-p

d= error at 95% confidence interval (5%)

d= 0.05

$$n = \frac{(1.96)^2 \times 0.5 \times 0.5}{(0.05)^2}$$

$$n = \frac{0.9604}{0.0025}$$

$$n = 384.16$$

Ethical Considerations

Ethical approval was obtained from ethical committee of the Jodhpur Dental College General Hospital (JDCGH), Jodhpur**.**

Study Population

A cross-sectional descriptive analysis was done on the environments of three selected departments of Jodhpur Dental College General Hospital (JDCGH), Jodhpur.

1. Oral and maxillofacial surgery: functions in extraction of mobile or meaningless teeth, orthodontic extraction, surgical correction of any maxillary and mandibular bone fracture or extraction of pathology.
2. Conservative and endodontics: is concerned with removal of caries from tooth surface and restoration, root canal treatment.
3. Periodontology and implantology: Is concerned with plaque control,scaling root planning, management of periodontin-Endodontic flap surgery, treatment of periodontium related disease and implant application procedures

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Collection, Transport and Analysis of Specimens

Air and surface samples were taken before and after dental procedures from all the three departments within durations of 14 days. Different antiseptics were used so as to compare their effectiveness.

a. Air Sampling

This was performed with settle plate method. Petri dishes containing nutrient agar were transported to the area in sealed plastic bags. The plates were labeled with sample number, site within the region, time and date of sample collection. The plates were placed at different chosen places in the room and exposed for 60 minutes. After this exposure, the plates were covered with their lids and taken to laboratory in sealed plastic bags and incubated at 37□C for 24hours.

b. Surface Sampling

A swab soaked in nutrients broth was used to collect samples from the floor, walls, dental chairs, dental stand and light handles. All samples were properly labeled and immediately transported to the microbiology laboratory of the General Hospital within the campus.

c. Processing of Samples

Swabs taken from different places were streaked on blood agar plates and incubated at 37^{o}C under aerobic conditions for 24 hours. After incubation, isolates obtained were appropriately identified. After aerobic incubation of the settle plates at 37^{0}C for 24hours, the colonies on each plate were counted and recorded as the number of bacteria carrying particles settling over the area of the plate in a given period of time. The level of bacterial contamination of air is usually expressed as the number of bacteria carrying particles per cubic millimeter. BCP load in the environment was determined by a formula based on the colony count, area of the plate exposed, and the duration of exposure. The number of BCP settling on 1 m^2 of medium per minute is equal to the number of such particles per 0.3 cubic meter of air. Since the agar plate is 10 cm in diameter (i.e. 5 cm radius), its area (pr^2) is approximately equal to 78 cm^2, i.e., 0.0078, m^2. The acceptable upper limit of bacteria is 180 BCP/$m.^2$ this equals 54 BCP/0.3 m^2. Therefore, 1 m^2 of agar medium exposed for one minute should not have more than 54 colonies. Alternatively,

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0.0078 m^2 exposed for 60 minutes should not yield more than 54 x 60 x 0.0078, that is, 25.3 colonies.[12,17]

d. Antibiotic Sensitivity Test

The suspected or established pathogens isolated during the culture were subjected to antibiotic sensitivity testing using Kirby-Bauer method.

e. Efficacy Testing of Disinfectants

This was carried out in two ways, disc diffusion test and in-use test.

1. Disc diffusion test: This is suitable for comparism of a number of disinfectants, not for industrial use.
    - Sterile suspension of each organism was made using sterile distilled water and was matched with 0.5McFarland standard.
    - A lawn culture of the suspension was made on tripticase soy agar using sterile cotton swab.
    - 6mm sterile filter paper was soaked in individual dilutions of the disinfectant and was placed on the inoculated agar.
    - The plates were then incubated for 24 hours at 37^0C.
    - Following incubation, the diameter (in mm) of the zone of inhibition (which is as a result of the diffusion of disinfectant out of the disk and into the agar) was measured.
2. In-use testing for disinfectant: 1:10 dilution was made by mixing 9ml of sterile nutrient broth with 1ml of already used water-disinfectant mixture which was obtained after moping of floors of the different study units. A pair of nutrient agar plates are used for each unit, 10 drops of the mixture is aseptically placed on each plate. One was incubated at 25^0C and the other at 37^0C growths were observed within 24hours on all plates.

Results

Surface Assessment of a total of 274 surface samples were processed, 255(93.1%) were culture positive for bacteria and 19(6.9%) had no growth. Five different bacteria were isolated from all

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surfaces studied. One hundred and thirty-two (132) surface samples were collected in the morning out of which 126(95.4%) had growth with with only 6(4.6%) having no growth. Whereas in the afternoon, 142 were collected out of which 129((90%) had growth. The distribution of bacterial isolates from dental chairs, dental stands, light handles, dental floors and walls are represented on Tables below.

Air Assessment

A total of 97 air samples were processed and all samples were culture positive for bacteria. Higher bacterial contamination was caused by Bacillus spp. (35.7%) from all the 3 units similar to the result of Singh *et al.*,.[19]

Table 1 Showing Overall Distribution of Bacterial Isolates with Respect To Various Surfaces

Sample site	**Coagulase negative staphylococci N(%)**	***Pseudomonas aeruginosa* N(%)**	***Micrococcus luteus* N(%)**	**Bacillus spp. N(%)**	**Aerobic Spores N(%)**	**Total N(%)**
DC	41(7.1)	3 (0.5)	32(5.5)	57(9.8)	13(2.2)	146(25.1)
DS	27(4.6)	3(0.5)	26(4.5)	55(9.5)	12(2.1)	123(21.2)
LH	13(2.2)	1(0.2)	12(2.1)	47(8.1)	5(0.9)	78(13.4)
DF	35 (6.0)	5(0.9)	26(4.5)	58(10.0)	24(4.1)	148(25.5)
DW	9(1.5)	2(0.3)	4(0.7)	42(7.2)	29(5.0)	86(14.8)
Total	125(21.5)	14(2.4)	100(17.2)	259(44.6)	83(14.3)	581(100)

Keys: DC- Dental chair; DS- dental stand; LH- light handle; DF- dental floor; DW- dental walls

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Table 1 represents an overall distribution of bacterial isolates with respect to various surfaces. A total of 581 isolates were obtained from the positive bacterial cultures. Bacillus spp. is the most predominant organism (44.6%), followed by coagulase negative staphylococci (21.5%), *Micrococcus luteus*(17.2%), Aerobic spores (14.3%) and *Pseudomonas aeruginosa* (2.4%). Dental floor has the highest rate of contamination 25.5% (148 isolates), followed by dental chair 25.1% (146 isolates), dental stand 21.2% (123 isolates), dental walls 14.8% (86 isolates) and light handles 13.4% (78 isolates),

Table 2 Showing Overall Distribution of Bacterial Isolates with Respect to Time of Sample Collection

Sampling time	**Coagulase negative staphylococcus N(%)**	***Pseudomonas aeruginosa* N(%)**	***Micrococcus luteus* N(%)**	**Bacillus spp. N(%)**	**Aerobic Spores N(%)**	**Total**
Morning	63(10.8)	8(1.4)	48(8.2)	123(21.2)	36(6.2)	278(47.8)
Afternoon	62(10.7)	6(1.0)	52(9.0)	136(23.4)	47(8.1)	303(52.2)
Total	125(21.5)	14(2.4)	100(17.2)	259(44.6)	83(14.3)	581(100)

Table 2 represents the distribution of bacterial isolates with respect to time of sample collection. A total of 581 isolates was obtained out of which 303(52.2%) were isolated in the afternoon while 278(47.8) were isolated in the morning. Bacillus spp. has the highest prevalence, 44.6%.

Table 3 Showing Descriptive Values of CFU Count From Morning Samples Obtained In The Three Different Units.

		Minimum	maximum	Mean	Std. deviation
OS	No. of colonies	117	373	212.14	92.701

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	Calculated CFU/m^3	54.8	174.6	99.3	97.008
PI	No. of colonies	32	453	133	107.124
	Calculated CFU/m^3	15	212	62.24	50.136
CE	No. of colonies	138	409	229.06	69.769
	Calculated CFU/m^3	64.6	191.40	107.20	32.645

Key: OS- Oral Surgery; PI- Periodontics and Implantology; CE- Conservative and endodontics unit

Table 3 shows the descriptive values of CFU count from morning samples obtained in the three different units. All units exceed the acceptable limits of isolates except the minimum value obtained from periodontics and implantology unit.

Table 4 Showing Descriptive Values Of CFU Count From Afternoon Samples Obtained In The Three Different Units.

		Minimum	maximum	Mean	Std. deviation
OS	No. of colonies	37	213	101.39	52.518
	Calculated CFU/m^3	17.3	99.7	47.45	24.583
PI	No. of colonies	26	133	68.17	30.247
	Calculated CFU/m^3	18.2	62.2	31.90	14.153
CE	No. of colonies	41	221	99.18	56.254
	Calculated CFU/m^3	19.2	103.4	46.41	32.645

Key: OS- Oral Surgery; PI- Periodontics and Implantology; CE- Conservative and endodontics unit

Table 4 shows the descriptive values of CFU count from afternoon samples obtained in the three different units with only the minimal values in all three units falling within the acceptable limits of the bacteria carrying particles.

Table 5 Shows the Effect of Bleach on the Various Organisms at Different Concentrations.

	Varying concentrations of bleach/zones of inhibition (mm)

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Isolates	0.5%	1%	2%	5%	10%
Pseudomonas aeruginosa	0	0	0	0	8
Aerobic spores	0	0	0	0	0
Coagulase negative staphylococcus	0	0	0	0	0
Micrococcus luteus	10	12	13	15	15
Bacillus spp.	0	0	0	0	0

Table 5 shows the effect of bleach on the various organisms at different concentrations, *Micrococcus luteus* appears to be the only organism that succumbs to the effect of bleach at all concentrations.

Table 6 Shows Antimicrobial Susceptibility Patterns Of *Pseudomonas Aeruginosa* And Coagulase Negative Staphylococcus (Cons)

Pseudomonas aeruginosa		**Coagulase negative staphylococcus(CoNS)**	
Anti-biotic(concentration in µg)	Zones of inhibition (mm)	Anti-biotic(concentration in µg)	Zones of inhibition (mm)
Meropenem (10)	32	Vancomycin (30)	28
Cefepime (30)	25	Clindamycin (10)	0
Imipinem (10)	26	Linezolid (30)	29
Gentamicin (30)	24	Gentamicin (30)	25
Ciprofloxacin (30)	31	Erythromycin (10)	26
Amikacin (30)	20	Oxacillin (10)	32
Ceftazidime (30)	15	Tetracycline (30)	24

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Table 6 shows the Antimicrobial susceptibility pattern for *Pseudomonas aeruginosa* and Coagulase negative staphylococcus. Meropemem being the most sensitive thus having the highest zone of inhibition and ceftazidime being least sensitive for *p.aeruginosa*. And oxacillin resulted in the highest zone of inhibition for CoNS while it was resistant to clindamycin.

DISCUSSION

Samples were collected in the morning prior to dental procedures and in the afternoon after dental procedures. Increased rate of contamination was seen in samples collected in the morning [24]. This may indicate poor disinfection by sub-staff or ineffective disinfectant usage.

The bacteria isolated in air were similar to those isolated from surfaces which were *Bacillus spp*., coagulase negative staphylococcus, *Micrococcus luteus* and *Pseudomonas aeruginosa*. Same organisms were similarly isolated in a study conducted by Ghadah [20] on the prevalence and investigation of bacterial contamination in dental healthcare associated environment.

Bacillus spp. had the highest percentage of occurrence in air samples [19] and surface samples, 35.7% and 44.6% respectively. Unlike in a study on investigation of microbial contamination in the clinic and lab of prosthodontics department of dental school by Taheri [25] where *S. aureus* had the highest bacterial contamination (18.0%).

There was higher level of microbial contamination in the afternoon than in the morning. This finding is similar to that of Hoshyari *et al* [21]on evaluation of bacterial contamination in clinical environment of Sari dental school.

Coagulase negative staphylococcus, *Micrococcus luteus* and Staphylococcus aureus were the commonest isolates among the nine bacteria species identified in an investigation of level of air-borne pathogens in selected hospitals of Zarqa city, Jordan .[23] This is relatively similar in the findings of Alireza et al, [1] where Coagulase negative staphylococcus was the most predominant isolate.

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Antimicrobial susceptibility testing done on *P. aeruginosa* and Coagulase negative staphylococci was sensitive for all antibiotics employed. This shows that the organisms are new in the environment and needs prompt elimination to avoid emergence of drug resistant strains

Conclusion

With the current finding, it is an established fact that microbial contamination of surfaces and air of dental units exist and this necessitates the need for health professionals and students of dentistry to come up with more effective ways to curb cross-infection .[22] Inappropriate and/or in-effective environmental disinfection is a contributory factor in the rise in microbial load in the healthcare setting.

Lack of a universal procedure for surveillance of nosocomial infection, presence of pathogenic bacteria, poor hand hygiene and heavy contamination of some important surfaces are the most important problems in our hospitals.[1]

Recommendation

Reducing foot trafficking, improving the ventilation system and effective routine cleaning has to be made to maintain the aerobic bacteria load within optimal level .[8] Dental practices should develop a written infection-control program to prevent or reduce the risk of disease transmission. The program should outline the policies, procedures, practices, technologies, and products used to prevent occupational injuries and illnesses among dental team members as well as healthcare-associated infections among patients .[5] Further studies including mycology should be conducted to determine the optimal intervention to reduce microbial contamination in the hospital environments.

References

1. Alireza, E., Kayedani, A., Jahangir, M., Kalantar, E. and Jalali M. (2010). Isolation of common aerobic bacterial pathogens from the environment of seven hospitals, Ahvaz, Iran *Jundishapur Journal of Microbiology* Vol. 4 No. 2, pp. 75-82.

2. Atlas R.M (1995). Microorganisms and human diseases. *Mosby-Year book*, Inc.

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3. CDC (2002). Guideline for hand hygiene in health-care setting: Recommendation of the health care infection control practices advisory committee and the HICPAC/SHEA hand hygiene task force. *Morbidity and Mortality Weekly Report*, 51: 1-4.

4. Chuaybamroong, P., Choomseer, P. and Sribenjalux, P. (2008). Comparison between hospital single air unit and central air unit for ventilation performances and airborne microbes. *Aerosol and Air Qualification Research*, 8(1): 28-36.

5. Dickson, S. K. and Bebermeyer, R. D. (2013). Guidelines for infection control in dental health care settings www.dentalcare.com/en-US/dental.education/continuing-education/ce90/ce90.aspx disinfection of bronchoscopes, contaminated with mycobacterium tuberculosis and mycobacterium aviun-intracellulase in sputum. *Journal of Hospital Infection* 45; 278-282

6. Friberg, B., Friberg, S., Burman, L. G. (1999). Inconsistent correlation between aerobic bacterial surface and air counts in operating rooms with ultra clean laminar airflows: Proposal of new bacteriological standard for surface decontamination *Journal of Hospital Infections* 42:287-293.

7. Friberg, B., Friberg, S., Burman, L. G., (1999). Correlation between surface and air counts of particles carrying aerobic bacteria in operating rooms with turbulent ventilation: an experimental study *Journal of Hospital Infections* 42:61-68.

8. Genet, C., Kibru, G. and Tsegaye, W. (2011). Indoor air bacterial load and antibiotic susceptibility pattern of isolates in operating rooms and surgical wards at Jimma university specialized hospital, southwest. *Ethiopian Journal of Health Science*. 21(1):9-17.

UNDER PEER REVIEW

9. Hambraeus, A. (1988). Aerobiology in the operating room–a review. *Journal of Hospital acquired Infection*. 11(supplement A): 68-76.

10. Jarvis, W.R. (2007). The inanimate environment In: Bennett JV, Brachman PS (eds), *Hospital infections*, 5th ed. Philadelphia, PA, Lippincott-Raven; 275-302.

11. Kampf, G. and Kramer, A. (2004). Epidemiologic background of hand hygiene and evaluation of the most important agents for scrubs and rubs *Clinical Microbiology Review* 17: 863-93.

12. Kelkar, U. Kelkar, S. Bal Abhijit, M. Kulkarni, S. Kulkarni, S. (2003). Microbiological evaluation of various parameters in ophthalmic operating rooms. The need to establish guidelines *Indian Journal of Ophthalmology* 51: 171-6

13. Lwanga S. and lemeshow S. (1991). Sample size determination in health studies: A practical manual medicine

14. Maki, D. G., Carla J. Alvarado, Carol B.S, Carol A. Hassemer, B.S. and Mary Ann (1982). Relationship of the inanimate hospital environment to endemic nosocomial infection. *National English Journal of Medicine*; 307:1562-1566

15. McCarthy, G. M., Sali, C. S, Bednarsh H, Jorge J, Wangrangsimakul K, Page-Shafer K. (2002) Transmission of HIV in the dental clinic and elsewhere. *Oral Disease* 8(Supplement 2):126–135.

16. Obbard, J. P. and Fang, L. S. (2003). Air-borne concentrations of bacteria in a hospital environment in Singapore Water, Air, and Soil Pollution 144:333-341.

UNDER PEER REVIEW

17. Prathab, A. G. and Lalitha, C. (2012). Microbiological surveillance of air quality in operation theatres - comparison of the conventional settle plate techniques versus use of an air sampling device *Journal of evolution of medical and dental sciences*/volume1/ issue4/October - page 371-381

18. Sarmukaddan S.B and Garad S.G (2006). On validity of assumptions while determining sample size. *Indian journal of community medicine* 29(2):204.

19. Singh, K., Dar, F. A. and Kishor, K. (2013). Bacterial Contamination in Operating Theatres of District Hospital Budgam in Kashmir Division. *Innovative Journal of Medical and Health Science* Vol. 3 No.2 pp62 – 63.
20. Ghadah Salim Abusalim (2022). Prevalence and investigation of bacterial contamination in dental healthcare associated environment. *Journal of king Saudi University-science* vol.34, issue 6, August 2022, 102153.

21. Hoshyari N, Allahgholipour Z, Ahanjan M, Moosahzadeh M (2018). Evaluation of bacterial contamination in clinical environment of Sari dental school. *Journal of Research in dentomaxillofacial science*. 2019; 4(2):19-25 DOI: 1029252/jrdms

22. Victor Hugo Marques Coellio, Gisely Naura Venancio, Thiago Fontanella Cestari, Maxine Ennata Alves De Almeida,Carolinie Batista Nobre Da Cruz (2016). Microbial contamination of a university dental clinic in Brazil. *Brazilian Journal of oral science*. October/December 2016-vol.15 number 4

23. Qudiesat, K.; Abu-Elteen, K.H.; Elkarmi, A.Z.; Hamad, M.; Abussaud, M. Assessment of airborne pathogens in healthcare settings. *African Journal of Microbiology Research.* 2009, *3*, 66–76. Available online: https://www.academicjournals.org/ajmr (accessed on 10 October 2021).

UNDER PEER REVIEW

24. Fekadu, S. and Getachewa, B. (2015). Microbiological assessment of indoor air of teaching hospital wards: a case of Jimma University Specialized Hospital. *Ethiopian journal of health sciences* 25(2):117-122.

25. Sharzad Taheri, Ghazaleh Shahabinezhad, Molook Torabi, Soodabeh Torabi Parizi. Investigation of microbial contamination in the clininic and laboratory of the prosthodontics department of dental school. *Pesqui Bras Odontopediatria Clinical Integration*. 2021;21:e0014.

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