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Microbial Contamination of the Mouth Masks Used By Post-Graduate Students in a Private Dental Institution: An In-Vitro Study

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Abstract:
Background and Aim: *Mouth mask is an essential infection control barrier used by the Dental health care professionals to prevent the Bioaerosols produced in the Dental Setup which are hazardous and a potent source of cross-contamination. This study aims at assessing, identifying and comparing the microbial contamination of the mouth masks used by post-graduate students of different departments in a Dental Institution.*
Materials and Methods: *A cross-sectional survey was conducted using a questionnaire on 36 post-graduate students from 9 departments of a private Dental Institution, and the samples of used mouth masks were obtained from them. The in-vitro analysis involved inoculation of a section of mouth mask from the external and internal surfaces in an enrichment media for isolation of bacteria and successive isolation by spread plate method on selective and non-selective media. Screening for Candida species was done by contact method on Candida agar.. Chi-square test, Mann-Whitney test and Kruskal - Wallis Test were used for statistical analysis.*
Results: *The external surfaces presented maximum bacterial load with the mean value of 31.7 x 10^2 cfu/ml (Department of Endodontics) and maximum mean value of 22.8 x 10^2 cfu/ml for internal surfaces (Department of Oral Medicine and Radiology) among all the departments. The fungal isolates (6 %) included strains of Candida species. The presence of other fungal organisms like* ***Aspergillus spp, Penicillium spp, Cladosporium spp*** *and* ***Alternaria spp*** *indicates air borne contamination.*
Conclusion: *The study proves that Mouth mask is a major source of contamination and Nosocomial infections and following a proper infection control protocol is necessary.*
Keywords: *Cross-contamination, Dental institution, Microbial contamination, Mouth masks, Post – Graduate students.*

I. Introduction

Historically, surgical attire and the ritual of gloving, gowning and donning of masks dates back to the 1860s [1]. In 1897, Fluegge demonstrated that ordinary conversation could disseminate bacteria-laden droplets from the nose and mouth, substantiating the need for an effective face mask. This marked the realization of the danger of human exhalation as a cause of surgical wound sepsis. In 1915, Weaver and Capps confirmed that face masks are effective against contagious diseases as well as cross-infection [2]. The face masks have now become an integral part of the personal protective equipments of the medical, dental and all the other health care providers [3].

From time to time, patients who are unwell may attend at a dental office. Their health condition may relate to a dental problem, such as an oral infection or a postoperative complication, but it may also relate to a non-dental problem, such as a severe respiratory illness (e.g. influenza) or simply a bad cold [2]. There exists a constant threat of infections and cross-contamination in the Dental operatory. Biological hazards, to which dental personnel are exposed in a dental office include pathogenic microorganisms (viruses, bacteria and fungi), allergic and toxic substances micro-organisms produce [4]. During a Dental procedure, the Dentist, patient and the dental staff are prone to be affected by cross- contamination and Nosocomial infections by a variety of infectious agents from the patients, staff, operating instruments, visitors, ventilation and air-conditioning systems, and even from the environment.

Micro-organisms can spread easily in closed spaces such as dental operatories, where every procedure performed in the oral cavity of a patient contaminates not only the instruments used in treatment but also the

hands of a dentist, the operative field, objects and surfaces in the close and more distant vicinity of a dental unit, and the air itself [4]. Bioaerosols formed from specific dental equipment usages, such as hand pieces, ultrasonic scalers and air polishers are composed of blood, calculus, saliva, plaque, nasopharyngeal secretions and so on and contain particles with less than 50 micrometer in diameter. These are invisible to the naked eye and can remain in the environment as aerosols for long periods of time. These aerosols may be inhaled into the lungs to reach the alveoli or may come in contact with the skin or mucous membranes [5]. Aerosol that are 100 micrometer or more in diameter are thought to be too large to be inhaled; however, they may still come into contact with the skin, eyes, and mucous membranes or may settle down on the exposed hair and clothing. Thus, diseases like pneumonia, influenza, hepatitis, and skin and eye infections may be transmitted during dental treatment procedures. At present, the most serious diseases threatening dentists and their staff are Hepatitis B and Acquired immunodeficiency syndrome (AIDS) [5].

Minimizing the risk of postoperative infections is the responsibility of all who work in the operating theatre (surgeons and nursing staff). In order to minimize this risk, dental operating personnel are required to follow accepted infection control practices. One well-entrenched infection control measure in the operating theatre is the routine use of surgical face masks. The Association of Operating Room Nurses (AORN) recommends that "all persons entering restricted areas of the surgical suite should wear a mask when open sterile items and equipment are present [6]. Therefore, dental practitioners and clinical auxiliary staff must wear suitable fluid-resistant surgical masks that block particles of 3 microns or less in size. Since masks protect the mucous membranes of the nose and mouth, they must be worn wherever there is a potential for splashing, splattering of blood, saliva or body fluids, or where there is a probability of the inhalation of aerosols with a potential for transmission of airborne pathogens.

Surgical masks for dental use are fluid-repellent paper filter masks and are suitable for both surgical and non-surgical dental procedures that generate aerosols. Masks supplied for use in dental practice are required to conform to AS 43814. This three-ply material is made up from a melt blown material placed between non-woven fabrics. The melt-blown material acts as the filter that stops microbes from entering or exiting the mask. Most surgical masks feature pleats/folds commonly three pleated are used allowing the user to expand the mask so it covers from the nose and under the chin [7].

According to the CDC guidelines, surgical mouth mask is a personal protective barrier [8]. The use of surgical face masks is synonymous with acute health care and is so deeply ingrained that to question it would have been unheard of until recently [9]. Unlike the white coats, the filtration abilities of a mask begins to decline after approximately 20 minutes with exposure to moisture and the external surface of a mask gets contaminated by the aerosols present in the environment and becomes a source of cross contamination and thus requires proper disposal. The following study was an attempt to address the lack of literature regarding contamination of mouth-masks in Dental settings. The purpose of the study was to present a comprehensive and comparative analysis of the microbiological flora harboring the mouth masks of dental personnel from different departments of a dental college and hospital.

The present study was conducted in a private Dental institution of Bangalore, India. Situated on the outskirts of the city, it provides Oral Care to a large population. Each department of the institution provides education at the post graduation level and the post graduate students are the ones who are exposed to the dental environment for the longest period of time as they provide Dental treatment to the patients on a frequent basis. This study was conducted on the Final year post graduate students of all the departments of a private Dental Institution to assess their practice of the infection control protocols. The study was approved by the Ethical Review Board of Rajarajeswari Dental College and Hospital, Bangalore.

II. Materials And Methods

2.1. A cross-sectional survey was conducted on 36 post graduate students consisting of 4 participants from each of the 9 departments of a private dental Institution of Bangalore, India, using a pretested, self administered questionnaire. The questionnaire, consisting of 19 questions was used to assess the knowledge and practice of the participants regarding their use of the personal protective barriers. It consisted of demographic details and questions related to practice regarding the use of mouth masks, frequency of changing, its storage, exchange with colleagues, removal of mouth masks before or after a case or with gloved or ungloved hands and attempting any case without a mask. The knowledge related questions dealt with awareness of the post graduates regarding the deposition of bacteria on mouth masks, the surface which is most contaminated, its potential of causing cross-contamination or preventing aerosols during conversation and its disposal with household wastes.

2.2. Microbiological Study

Each participant was also provided with a sterile mouth mask for use during the dental treatment procedures and was later collected back in sterile zip lock pouches to be carried to the laboratory. The samples were sectioned in a laminar airflow chamber. 1 cm square each was cut from the nose bridge of the external

surface and the area adjacent to the oral cavity of the internal surface. The cut section was then inoculated into brain heart infusion broth and incubated at 37^{o} C for 24 hrs at 120 rpm for the Bacterial study. The broth culture was serially diluted and 0.1 ml of this serially diluted sample was taken and spread plated Nutrient agar, Mannitol Salt agar, Mac Conkey agar, and Blood agar. These plates were incubated at 37^{o} C for 24 hrs. For the Fungal culture the cut sections were pressed on the surface of Candida agar media for 5 minutes and removed later. The agar plates were incubated at 28^{o} C for 2-3 days.

A colony counter was used to count the bacterial colonies present on the nutrient agar and was expressed in terms of cfu/ml. Biochemical tests were performed for colonies isolated on each agar media for the identification of the pathogens.

Descriptive statistics were used to describe the number of colony forming units in each department. ANOVA and Kruskal-Wallis test were used to evaluate the significance of the observed differences in the cfu/ml recorded from the participants of each department. Chi square test was used to evaluate the responses of the participants to the questionnaire. The level of significance was considered to be $p < 0.05$.

III. Results

Out of the total 36 participants, 17 were males and 19 were females. Analysis of the responses expressed in the questionnaire revealed that while majority of participants wear their mouth-masks chair-side and about 16 % wear them all the time. About 20% have the practice of putting on their mouth-mask after wearing their gloves and 72 % remove the mouth-mask with their gloves on. 47% of the participants store their mouth masks in the instrument trays, while 44% in their white coat pockets. Although a large number of participants believed that mouth-mask can cause cross-contamination when touched, 46% have the habit of removing their mouth-mask after they have washed their hands. Surprisingly, 33% responded that they have attempted cases without wearing a mouth-mask and 55 % to have contacted/removed their mouth-mask after they have started a case. About 22 % stated that they have exchanged their mouth-masks with others. 13 % of participants indicated that a mouth-mask can be disposed along with normal household waste.

Table 1. Responses of the Participants According to the Variables

Variables		Results %
Gender	Male	17
	Female	19
Duration of wearing mouth mask	Only chair side	83.3
	All the time	16.7
Protective barrier for each case	Mouth mask	19.7
	All	69.4
	Mouth mask and Gloves	5.6
	Mouth mask, head cap and Gloves	5.6
Frequency of changing/disposal	Every case	63.9
	Once daily	33.3
	Once a week	2.8
Practice of exchanging	Yes	22.2
	No	77.8
Storage of mouth mask	White-coat pocket	44.4
	Books	2.8
	Office desk	5.6
	Instrument Tray	47.2
Case attempt without mouth mask on	Yes	33.3
	No	66.7
Awareness regarding used mouth-mask causing cross contamination if touched	Yes	94.4
	No	5.6
Disposal of mouth mask with house hold waste	Yes	13.9
	No	86.1
Removal of mouth mask	With gloves On	72.2
	Without gloves	27.8
	After washing hands	8.3
	Before washing hands	91.7
Hygiene practices	Wear mouth mask before wearing gloves	80.6
	Wear mouth mask after wearing gloves	19.4
	Removing the mask for conversation in between a case	55.6

Table 2: Microbial load on the different surfaces of the mouth mask in terms of cfu/ml as found on Nutrient agar.

Department	Internal Surface	External Surface
Prosthodontics	14.59×10^2	5.76×10^2
Pedodontics	13.19×10^2	17.80×10^2
Oral Medicine and Radiology	22.82×10^2	6.86×10^2
Endodontics	15.09×10^2	31.70×10^2
Orthodontics	17.92×10^2	11.10×10^2
Oral Surgery	13.41×10^2	23.18×10^2
Public Health Dentistry	4.31×10^2	7.13×10^2
Oral Pathology	6.67×10^2	6.64×10^2
Periodontics	22.42×10^2	21.22×10^2

TABLE 2 shows the mean values of the colony forming units/ml for the external and internal surfaces of the mouth masks collected from the post graduate students of different departments. The highest microbial count on the external surface was seen in the Department of Endodontics with an average count of 31.70×10^2 CFU/ml and the least was seen in the Department of Prosthodontics with an average count of 5.76×10^2 CFU/ml. The highest count on the internal surface was seen in the Department of Oral medicine and Radiology with an average count of 22.82×10^2 CFU/ml and the least was seen in the Department of Public Health Dentistry with an average count of 4.31×10^2 CFU/ml. The microorganism isolated from different culture media plates accounted for E.coli (54%) , S.aureus (25%) , Micrococcus (1%) , Klebsiella (5%), Enterobacter (2%), Pseudomonas (3%), Enterococcus (4%), Candida (6%). **(Fig 1).** It is to be noted that the control mouth mask showed the presence of E. coli**,** which however was insignificant when compared to those present on the used mouth masks.

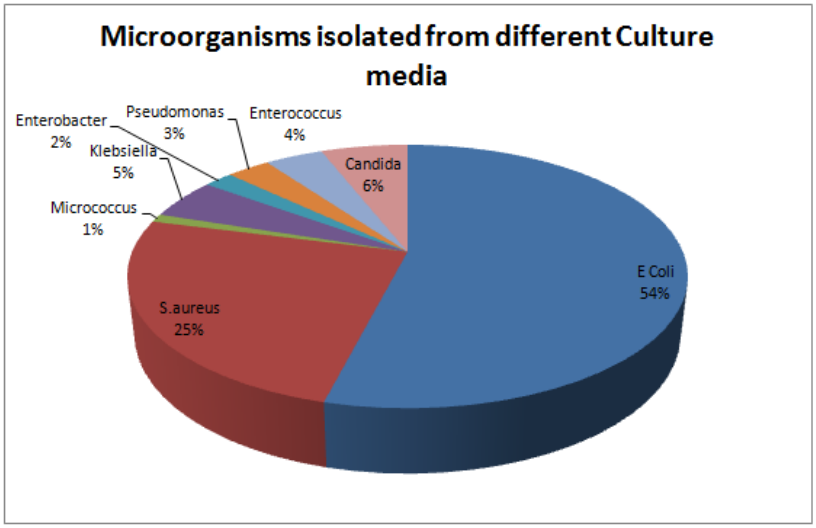

Figure 1 : Microorganisms Isolated from Different Culture Media

IV. Discussion

The study was done to find the risk assessment on the usage of mouth masks by the post graduate students belonging to different departments in a private Dental Institution. The study included a cross-sectional survey using a questionnaire, followed by microbiological study of the used mouth masks to assess, identify and compare the microbial contamination of the same. A significant chi-square value at $p< 0.05$ was obtained for the responses to the questionnaire regarding improper usage, handling and storage of the mouth masks.

Analysis of the responses to the questionnaire revealed that the participants were not completely aware of the protocols on the proper usage of the mouth masks. Although most of the participants have professed to having changed their mouth mask after each case, the microbial load on the surfaces otherwise seems to be high. This could possibly be due to external contamination generated during the dental treatment from air source, storage of the mouth mask like keeping it in white coat pockets or in their instrument trays and also regular handling of the masks for adjustments and during conversation with the supporting staff or the patients.

In present study it was found out that 47% of the participants store their mouth masks in the instrument trays and 44% of them store it in their white coat pockets. Previous studies have revealed that white coat pockets harbor pathogenic organisms which can be a source of Nosocomial infections [29]. This finding was in accordance with the findings of Varghese and Patel [37] where it was found that bacteria are most likely to be isolated from the pockets and sleeves of white coats since these are a source of Nosocomial infection. In our study, 46% of the participants reported to have removed their mouth masks after washing their hands which is a breach of the infection control protocol.

According to Larato et al [38] and Bennet AM et al [34], Bioaerosols which remain suspended in the Dental environment during and after the treatment procedures are a risk factor to acquire infections. In a study done on the risk of contamination of dentist's face by Nejatidanesh et al [36], it was found out that the areas around the nose and the inner corner of eyes are more contaminated than other areas of the face. In the current study, 33% of the participants responded that they have attempted cases without wearing a mouth-mask, and 55% have contacted/removed their mouth-mask after they have started a case putting themselves under the risk of being exposed to the environmental pathogens.

The microbiological study involved the culturing of the microbes present on the used mouth masks on different culture media. The two areas selected for the study were the external nose bridge area and the internal area adjacent to the oral cavity.

Among all the departments under study, Department of Oral Pathology showed equal contamination on both the external and internal surface and the microbial load was minimal. Among the remaining 8 departments under study, 4 of them showed high microbial load on the external surface. The departments included were Departments of Endodontics, Oral and Maxillofacial surgery, Pedodontics and Public health Dentistry. The highest contamination was seen in the Department of Endodontics with an average count of 31.70 $X10^2$ CFU/ml and the least was seen in the Department of Prosthodontics with an average count of 5.76 $X10^2$ CFU/ml. The high microbial load could be due to the increased amount of aerosols generated due to use of high speed airotors during the cavity preparation, a practice commonly undertaken in the Department of Endodontics.

The departments that showed high microbial load on the internal surface were Departments of Oral Medicine and Radiology, Periodontics, Orthodontics and Prosthodontics. The highest contamination was seen in the Department of Oral medicine and Radiology with an average count of 22.82 $X10^2$ CFU/ml and the least was seen in the Department of Public Health Dentistry with an average count of 4.31 $X10^2$ CFU/ml. Although the highest contamination in the internal surface is comparatively lesser than the highest contamination on the external surface, this could be attributed to improper usage and disposal of the mouth masks by the Post graduate students.

A similar study was conducted by Baratam et al in 2014[7], on the microbial contamination of mouth masks used by interns, post graduates and staff of all the departments of a Dental institution, where it was found that the Department of Oral Medicine and Radiology had the highest microbial count on the external surface and the Department of Periodontics had the highest microbial count on the internal surface. However, the least microbial count on the external surface was seen in the Department of Prosthodontics and that on the internal surface in the Department of Oral Pathology [7]. The findings of the lowest microbial count are similar to the present study.

To study the prevalence of any opportunistic pathogens the different special purpose media were used. On Mac Conkey agar, ***Klebsiella spp., E.coli, Enterobacter spp.*** and ***Pseudomonas spp*** were present. The presence of both ***E.coli*** and ***Pseudomonas spp*** needs attention since they are opportunistic pathogens have been implicated to cause nosocomial infections. ***E.coli*** and ***Pseudomonas spp*** *are* known to cause urinary tract infections, dermatitis, soft tissue infections, bacteremis, bone and joint infections, diarrhea, upper respiratory tract infections, osteomyelitis etc. ***Klebsiella spp and Enterobacter spp.*** are known to cause pneumonia, bloodstream infections, surgical site infections, endocarditis and intra-abdominal and pelvis infections, lower respiratory tract infections, septic arthritis, CNS and ophthalmic infections.

On Mannitol salt agar ***S.aureus*** and ***Micrococcus spp*** were present**.** These micro-organisms are known to cause skin infections, cellulitis, abscess, pneumonia, recurrent bacteremia, Nosocomial infections, septic shock, septic arthritis, endocarditis, meningitis, cavitating pneumonia, osteomyelitis, toxic shock syndrome, parotitis, mucositis, angular cheilitis, etc.

On Blood agar based on the haemolysis and biochemical test the organisms were identified as ***Enterococcus*** and Group D ***Streptococci***. These organisms are known to cause bacteremia, endocarditis,

hematogenous osteomyelitis, septic arthritis, pneumonia, urinary tract infection, meningitis, neonatal sepsis, bacterial peritonitis, vertebral osteomyelitis etc.

The commonly encountered bacterial pathogens like ***Escherechia coli***, ***Staphylococcus aureus*** indicate that clinically significant contaminants are harboring the mouth mask. This finding is similar to the findings of Baratam et al [27] where most dominant pathogens isolated were ***E.coli*** in 53% of the total samples and ***S.aureus*** in 23% samples.

Study of fungal contamination was also undertaken by making of impression of the mouth mask cut piece on Candida agar for selective isolation of Candida albicans. It is an opportunistic pathogenic yeast which causes Candidiasis, a systemic infection affecting oropharyngeal/eosophageal and genital/vulvovaginal tracts. 6% of the samples tested positive for Candida albicans. The presence of other fungal organisms like ***Aspergillus spp, Penicillium spp, Cladosporium spp*** and ***Alternaria spp*** indicates air borne contamination. These organisms cause aspergillosis, systemic penicilliosis, multiple brain abscess, invasive infections, fatal pneumonia, fungal meningitis, infections of the skin, eye, sinuses and brain.

In the study conducted by Baratam et al [7] it was found that, the mouth-masks worn by the postgraduate students showed the highest bacterial isolates and contamination in terms of the relevant pathogens suggesting that they are the high risk category among the dental personnel, bearing high risk for the event of cross-contamination in the dental setting. This finding supports the findings of the present study.

V. Conclusion

In the present day, the risk of health care associated infections and its transmission not only involves the people who are in direct contact with the patients but also those who work in such environments. Bioaerosols are omnipresent in a dental environment and increase significantly with increasing working hours thereby enhancing the risk of disease transmission. Most of the organisms isolated in this study from the mouth-masks were potentially pathogenic. Stringent measures needs to be implemented to halt and combat this alarming situation. Strict adherence to the infection control protocol, use of personal protective wear and its disposal must be followed by all those who work in the dental environment. Educating health professionals on the consequences of Nosocomial infections and advocating simple preventive measures such as hand-washing after each patient exposure and pre-procedural mouth rinsing of the patients before oral examination can limit the extent of cross contamination. Regular fumigation of the operating chambers may help to put a check on the airborne pathogens. The water and the suction units of the dental chair should be periodically cleaned to prevent any moisture borne pathogens from growing. Also, the Dental clinics should have adequate ventilation to prevent the accumulation of moisture. These simple preventive measures can be helpful in limiting the spread of Nosocomial infections to ensure a healthy environment for the patients as well as the health care providers.

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Conflicts of Interest

There are no conflicts of interest.

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