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Research Article

Instrumented Posterolateral Fusion inSpondylolisthesis

Identification and Quantification of Staphylococcus Aureus in Saliva at Different Time Intervals Among Dental Personnel Wearing Surgical Masks During Sars Cov2- A Prospective Observational Study

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Abstract: Our world combats the rapid spread of Covid 19 by wearing face masks which have become the norm. But it's effect on oral microbiome is yet to be studied. Staphylococcus aureus is the leading cause of bacterial co-infection in Covid-19-affected patients worsening their prognosis. Dislodgement of this bacterium from the oral cavity is said to cause many systemic diseases. As dentists are more prone to an aerosolic environment and a potential source of cross-contamination, we intended to identify and quantify S. aureus in the saliva of dental personnel wearing surgical masks. Unstimulated whole saliva samples of 120 healthy dental professionals wearing surgical masks were cultured for S. aureus growth in Mannitol Salt Agar medium for 48 hours at 37°C. After 1 hour and 6 hours of mask usage, the number of colony-forming units counted. After 1 hour and 6 hours of mask usage, 56% and 52.5% demonstrated growth of S. aureus respectively. There was a statistically significant mean reduction of 1.61*103 colony forming units after 6 hours of mask usage. There is a reduction in the prevalence and number of colony-forming units of S. aureus in the unstimulated saliva of healthy dental professionals after 1 hour and 6 hours of surgical mask use. As a result, mask use not only controls the spread of Covid but may also aid in reducing the source and spread of S. aureus cross infection, lowering the risk of morbidity and mortality in diseased patients.

Keywords: Covid, Face Mask, Saliva, Dentist, Bacteria and Staphylocoocus Aureus

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1. INTRODUCTION

The entire world is in the midst of a COVID-19 pandemic caused by a deadly virus, the SARS-CoV-2, which has been causing 508,041,253 infections with 6,224,220 deaths as on April 27, 2022[1]. Since, the virus spreads mainly through aerosol, standard operative protocols such as social distancing and face masks with modifications are practiced to contain the spread of the disease. A face mask acts as a physical barrier to the respiratory and muco-salivary droplets which may enter through the nose or mouth of the infected individuals who are shedding the virus[2,3]. Face mask not only protects us from the spread of the deadly virus, but it also affects the oral environment by increasing the humidity and carbon dioxide content. [4,5]This would have caused alterations in the oral microflora which is yet to be studied. The oral cavity is a diverse ecosystem that is home to a wide range of micro-organisms, viruses, and bacteria. Some of these micro - organisms are responsible for multiple infections. Because of the link with the external environment, oral flora is constantly changing and producing bacteriocin against one another to contend for nutrients in this small ecosystem. The oral cavity has many surfaces, each of which is completely covered with a slew of bacteria, the aforementioned bacterial biofilm. A number of these bacteria have been linked to oral diseases like caries and periodontitis, two of the most prevalent infections caused by bacteria. As the oral cavity comprises more than 700 colonizing species, it is too extensive and nearly impossible to study all the microorganisms.[6] One among them, Staphylococcus aureus was the most common pathogen that caused bacterial co-infections in past viral pandemics like Spanish flu. Even during this Covid 19 pandemic, it has been associated with increased morbidity and mortality.[7]Also, dental health professionals are exposed to aerosol during clinical practice and hence may act as a potential source of cross-contamination of these bacteria[8]. Hence, the effect of the usage of face masks on the occurrence of S. aureus in the oral cavity in dental health professionals is being determined.

2. METHODOLOGY

2.1 Selection Criteria

The present study was conducted in a hospital-based setup among healthy dental personnel in the region of Puducherry, after obtaining approval from the Institutional Review and Institutional Ethics Board. (IGIDSEIEC - 2021NRP34PGANOPM) A total of 120 healthy dental professional volunteers above 18 years of age were enrolled into the study by convenient sampling. Individuals with a history of any systemic illness, medications for the past 3 months, local infections in the oral cavity, and regular mouthwash users were excluded in the study.

2.2 Basic Protocol / Design

Details of participants including basic demographic data, history of brushing and tongue cleaning habits, and intraoral examination for oral hygiene status, caries, restoration, missing teeth, and prosthesis were recorded. Participants were asked to wear surgical masks EN 14683 Type IIR (Filtra) for a period of 6 hours. Samples were collected at two different time intervals i.e. after 1 hour (9:30 a.m.) and after 6 hours (2:30 p.m.) of wearing a surgical mask. Unstimulated whole saliva was collected in a sterile container, by spitting method and was processed immediately in the department of Microbiology. Using a micropipette, 1 microlitre of saliva sample was inoculated on the Mannitol Salt Agar plate, the selective media for S. aureus. The plates were incubated at 37°C for 48 hours. Colonies showing yellow pigmented growth surrounded by a yellow halo were recorded as S. aureus and the numbers of Colony Forming Units (CFU) were noted down (Figure 1, 2).

3. STATISTICS ANALYSIS

Descriptive statistics were performed using the SPSS software version (version 16, IBM, Corp. USA) with significant differences of $P \leq 0.05$ at a 95% confidence interval.

4. RESULT

Table 1: Demographic data

Total Samples	Age In Years		Gender	
	Range	Average	Male	Female
120	18 - 35	21	45(37.5%)	75 (62.5%)

Table 2: Age-wise Distribution of Colony Formation after 1 hour and after 6 hours of mask usage

Age in years	Colony Formation after 1-hour n (%)		Colony Formation after 6 hours n (%)	
	Present	Absent	Present	Absent
18	6 (50.0)	6 (50.0)	3 (25.0)	9 (75.0)
19	21 (80.8)	5 (19.2)	19 (73.1)	7 (26.9)
20	2 (11.8)	15 (88.2)	4 (23.5)	13 (76.5)
21	15 (61.2)	7 (31.8)	17 (77.3)	5 (22.7)
22	9 (52.9)	8 (47.1)	9 (52.9)	8 (47.1)
23	2 (33.3)	4 (66.7)	1 (16.7)	5 (83.3)
24	6 (60.0)	4 (40.0)	5 (50.0)	5 (50.0)
25	4 (66.7)	2 (33.3)	4 (66.7)	2 (33.3)
32	0 (0.0)	2 (100.0)	0 (0.0)	2 (100.0)
35	1 (50.0)	1 (50.0)	1 (50.0)	1 (50.0)

Table 3: Gender-wise Distribution of Colony Formation after 1 hour and after 6 hours of mask usage

Gender	Colony Formation after 1 hour n (%)		Colony Formation after 6 hours n (%)	
	Present	Absent	Present	Absent
Male	25 (55.6)	20 (44.4)	22 (48.9)	23 (51.1)
Female	41 (54.7)	34 (45.3)	41 (54.7)	34 (45.3)

Table 4: Comparison of the number of Colony-forming units after 1 hour and after 6 hours of mask usage

After 1 hour (CFU/ml)			After 6 hours (CFU/ml)			Mean Difference after 1 hour and after 6 hours (CFU/ml) by Paired t Test
Min.	Max.	Avg.	Min.	Max.	Avg.	
$0*10^3$	$40*10^3$	$7*10^3$	$0*10^3$	$35*10^3$	$5*10^3$	- $1.61*10^3$ ($P \leq 0.05$)

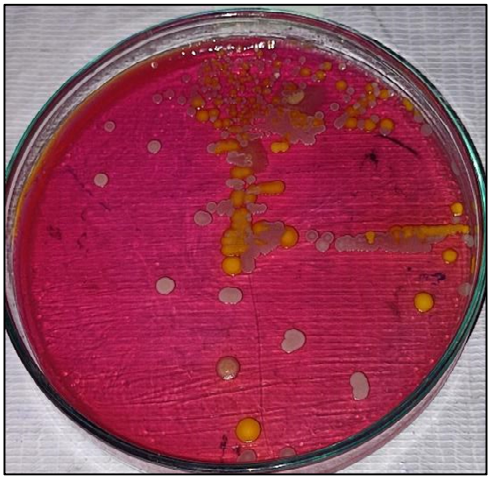

Fig 1: Demonstration of growth of S. aureus is seen as yellow colonies in mannitol salt agar plate during the 1st hour of mask usage.

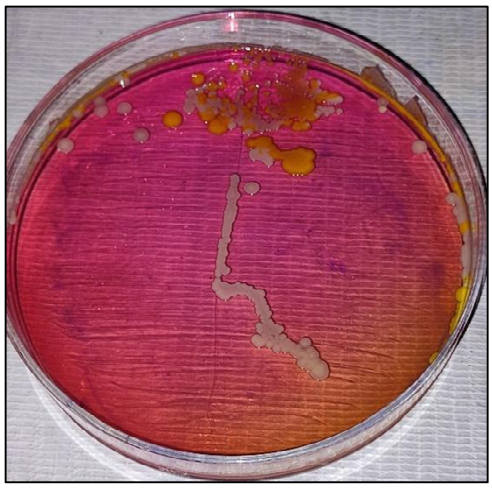

Fig 2: Demonstration of growth of S. aureus is seen as yellow colonies in mannitol salt agar plate during the 6th hour of mask usage of the same participant

5. DISCUSSION

At present, people worldwide are facing the era of a deadly pandemic caused by SARS-CoV-2. The morbidity and mortality of this viral infection are further increased by bacterial co-infection by Staphylococcus aureus. S. aureus plays an important role in diseases like Infective endocarditis, Bacterial pneumonia, bacteremia, rheumatic arthritis & in oral diseases like angular cheilitis, mucositis, sialadenitis, acute post-operative parotitis. Dislodgement of these bacteria from the oral cavity has also been documented to cause other systemic illnesses and antimicrobial paints have been described[8, 9]But the inhabitance of Staphylococcus aureus in the oral cavity is still controversial. It is questionable about the role it plays in the ecology of the healthy oral flora and the pathogenesis of oral and systemic diseases.[10]Dentists and the dental clinic environment being prone to aerosol exposure might act as a potential source of cross-contamination during this pandemic. The effect of usage of face masks on the occurrence of S. aureus in the oral cavity in dental personnel was studied which should be done by comparing with a control group without the usage of face masks. As it is unethical and difficult to find participants without face masks in the pandemic situation, we compared the count of S. aureus in the same individual at different periods after 1 hour of wearing a surgical mask and after 6 hours of wearing a surgical mask. Following the guidelines of WHO, our study participants (dental personnel) were advised to wear surgical masks EN 14683 Type IIR (Filtra) (protects from fluid splash, used in the operating room or procedural settings). [11]As Gideon J.W. et al in 2021 stated the mean duration of using the mask as 5.98 hours/day, we considered using surgical masks for a maximum time period of 6 hours. [12]. In our study, the participants were in the age group of 18-35 years with males 37.5% and females 62.5%. Although age plays an important role in microbiome dysbiosis, age-wise analysis of the difference in Colony Formation is not possible due to minimal samples of different ages[13]. According to the existing evidence, the prevalence of S. aureus in saliva relative to gender is not yet documented. Though studies have stated that gender can be assumed to be an important factor affecting the oral microbiome, [13] in our study gender-wise distribution of Colony Formation after 1 hour and after 6 hours of mask usage does not show a statistically significant difference. All the participants maintained good oral hygiene and brushing habits. Other findings like caries, restoration, missing teeth, and prosthesis were minimal/ negligible and hence not comparable as separate groups. After 1 hour of mask usage, 56% demonstrated growth of S. aureus. The isolation frequency in the present study is relatively higher as compared with previous studies that reported 46□ in saliva in healthy adults aged 22-43 years[15] and 20% in the saliva of students[14]. In another study, a relatively higher frequency of 60% in the saliva of healthy subjects [13, 16, 17]. The variations observed in the prevalence might be due to differences in the method of sample collection, culture media, and methodology used for the isolation of *Staphylococcus aureus*. After 6 hours of mask usage, unstimulated saliva samples demonstrated 52.5% demonstrated growth of S. aureus. This shows about a 2.5% reduction in prevalence which may be due to mask usage. Many researchers have studied the prevalence of S. aureus in the oral cavity but only Ohara-Nemoto et al., had quantified it stating that S. aureus

was the most frequent species occupying 46.4 % of the total number of bacteria (10^2 – 10^4 CFU/ml) in saliva. [15] In our study, Colony Forming Unit (CFU) formed were ranging from $0*10^3$ CFU /ml to $40*10^3$ CFU /ml with an average of $7*10^3$ CFU /ml after the 1 hour of mask usage. After 6 hours of mask usage, the CFU ranged from $0*10^3$ CFU /ml to $35*10^3$ CFU /ml with an average of $5*10^3$ CFU /ml. Paired t-test was conducted to compare the CFU after1 hour and 6 hours of wearing a face mask. After 6 hours of mask usage, the S. aureus count appears to be reduced by a statistically significant mean reduction of 1.61. The possible reasons for the decrease in CFU could be due to an increase in the humidity and carbon dioxide content in the oral environment on prolonged usage of a face mask which would have inhibited the growth of S. aureus[19]. Masks not only protect us from the spread of the deadly disease, but it may also aid in decreasing the incidence of many diseases like aspiration pneumonia, bacteremia, periodontitis, periimplantitis, angular cheilitis, parotitis, osteomyelitis by reducing the count of S. aureus[9,20,21, 22]It may also be useful to consider the usage of masks during this pandemic situation to avoid mortality and morbidity due to secondary bacteremia related illness. Even though there are few other techniques to counter bacteremia, the mask is the standard one[23]. Studies in the future without mask usage can be conducted to assess the quantity of S. aureus through which baseline data can be obtained. Also, the effect of mask usage by alteration in Staphylococcus aureus count in different illnesses can be studied.

6. CONCLUSION

There is a reduction in the prevalence and number of colony-forming units of S. aureus in the unstimulated saliva of healthy dental professionals after 1 hour and 6 hours of surgical mask use. As a result, mask use not only controls the spread of Covid but may also aid in reducing the source and spread of S. aureus cross infection, lowering the risk of morbidity and mortality in diseased patients.

7. AUTHORS CONTRIBUTION STATEMENT

SR, UDS, SM- concept and design, ANS, SM— data collection, VS, VDS- statistics and supervision, SDA– manuscript and communication

8. CONFLICT OF INTEREST

Conflict of interest declared none.

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