

Motorcycle Helmets as Potential Fomites in Transmission of Pathogenic Micro-organisms and Antibacterial Activity of Helmet Disinfectant Spray

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ABSTRACT

Helmets provide protection to our heads from grave injuries. Though they offer plenty of advantages, they act as breeding grounds for infectious pathogens. Despite the high possibility of being contaminated, helmets are seldom cleaned. There is less awareness among general public about transmission of pathogens through helmets and their disinfection process. The present study seeks to identify the types of bacteria and fungi contaminating helmets and to enlighten the participants about the good hygienic practices. In the present study, 30 bacteria were isolated and identified. Among the 30 isolates, 3 were found to be gram positive cocci in clusters (10%) followed by 8 gram negative rods (27%) and 19 were found to be gram positive rods (63%). A total of 23 fungi were isolated and identified in our study. Helmet cleaning and microbiological safety are key concerns for numerous and regular users. Spray disinfection solutions based on chemicals can be utilised nowadays. Antibacterial activity of a helmet disinfectant spray (Ustreaa) was done by both spread plate method as well as by disc diffusion method. The total colonies which developed following spread plate method for both before and after the application of a helmet disinfectant spray were observed to be 167 colonies and 91 colonies respectively. The antibacterial activity of a helmet disinfectant spray by disc diffusion method exhibited bactericidal activity by producing a zone of inhibition of 21mm.

Key words: Motorcycle Helmets, Bacteria, Fungi, Helmet disinfectant spray, Antibacterial activity.

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INTRODUCTION

Motorcyclists wear motorcycle helmets as a sort of protective headgear. A motorcycle helmet's main objective is to safeguard the rider's head following a collision, avoiding or decreasing head injuries and potentially protecting the rider's life. Motorcycle helmets, on the other hand, may pose a health risk to its wearers. Micro-organisms may be encountered almost everywhere and are an important element of every ecosystem. They exist freely or as parasites in various habitats. They exist as transitory contaminants

in fomites or hands in certain situations, posing serious health risks as reservoirs of community and hospital-acquired diseases. Continuous handling and usage of motorcycle helmets by various users might provide an ideal environment for the spread of germs and other microbes,^[1] amongst users, as well as the transfer of dangerous germs and infectious illnesses.

Human skin is continuously exposed to germs from the environment and is easily colonised by specific microbial species. Approximately 10 cfu/ml bacteria can be found on human skin.^[2] Diptheroides, Coagulase negative Staphylococcus, *Staphylococcus aureus*, Streptococcus, Bacillus spp., Candida spp., and *Mallassezia* spp are among the bacteria and fungi present on the skin's natural microbiota.^[1] Nevertheless, if found into unfamiliar surroundings or vulnerable individuals, such regular microbiota could cause illness.^[3] Other research has focused on the susceptibility of riders, passengers,

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and pedestrians,^[4] as well as variables influencing helmet use,^[5] helmet periodicity,^[6] and headgear public safety.^[6-8] There are very few studies related to helmets serving as vectors for transmitting pathogens and there is no study to the best of our knowledge on evaluating the efficacy of helmet disinfectant spray. Hence, this study was taken up to determine the micro-organisms associated with commercial motorcycle helmets used by college students in Chennai and to evaluate the efficacy of a helmet disinfectant spray.

MATERIALS AND METHODS

Sample collection and Processing

Forty samples (twenty for bacteria and twenty for fungi) were collected with the help of sterile swabs from the helmets used by the college students. The swabs were moistened with nutrient broth and normal saline to facilitate proper swabbing from the external and internal surface of the helmets. The samples were transported to the laboratory for further processing.

Isolation of bacteria

Twenty samples with nutrient broth were incubated at 37°C for 24 hrs. They were inoculated on to Nutrient Agar (NA), Mannitol Salt Agar (MSA), Blood Agar (BA) and Mac Conkey Agar (MAC). The plates were incubated at 37°C for 24 hrs. Next day the colonies were picked up and Gram staining, motility, catalase and oxidase were done. The bacterial isolates were identified as per the standard protocols.

Isolation of fungi

The fungal isolation was done with the other twenty samples with normal saline. They were inoculated on to Sabouraud Dextrose Agar (SDA) and the plates were incubated at 25°C to 35°C for a week. Identification of the fungus was done as per standard methods.

Antibacterial activity of a helmet disinfectant spray

a) Spread plate method

A helmet disinfectant spray Ustraa was used to assess its antibacterial activity on helmets. Ustraa helmet disinfectant spray was composed of butane, isobutane, propane, isopropanol. Two samples were collected from the helmet, one before the application of the disinfectant spray and one after the application of the disinfectant spray. The swabs were vortexed in 1ml nutrient broth for 2 min. 0.1ml was spread plated onto nutrient agar plates and was incubated for 24 hrs at 37°C. Next day the total colonies which developed were

counted with the help of colony counter and the results were recorded.

b) Antibacterial activity of a helmet disinfectant spray by disc diffusion method

Muller-Hinton agar (MHA) was poured into the Petri plates aseptically and was allowed to solidify. The lawn of the test bacterial strain was done with the help of sterile cotton swab. Sterile disc was impregnated with the disinfectant spray. It was placed onto MHA with sterile forceps. The test plates were incubated aerobically at 37°C for 24 hrs. After incubation, the results were recorded, as the presence or absence of inhibition zone. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the disc.

RESULTS

Prevalence of bacterial isolates from helmets

A total of 20 samples were collected from the helmets of college students and were processed, of which 30 bacteria were isolated and identified. Among 30 isolates, 3 were found to be gram positive cocci in clusters (10%) followed by 8 gram negative rods (27%) and 19 were found to be gram positive rods (63%). All the three-gram positive cocci isolated were found to be Coagulase negative Staphylococci (CoNS).

Prevalence of Gram-negative rods from helmets

8 gram negative rods were isolated from the helmets and these included *Leminorella grimontii*, *Enterobacter ascorbata*, *Hafnia alvei* and *Citrobacter rodentium*. (Table 1)

Prevalence of Gram-positive rods from helmets

19 gram positive rods were isolated from the helmets and these included the following - *Bacillus amyloliquefaciens*, *Bacillus acidicer*, *Bacillus cereus*, *Bacillus validus*, *Bacillus pumilus*, *Paenibacillus amylolyticus*, *Bacillus stearothermophilus*, *Bacillus sphaericus* and *Bacillus megaterium*. (Table 2)

Prevalence of fungal isolates from helmets

A total of 20 samples were collected from the helmets of college students and were processed, of which 23 fungi were isolated and identified. Among 23 isolates, the following fungi were identified which included

Table 1: Prevalence of Gram-negative bacteria (n=8).

S.No.	Name of the bacteria	Percentage of the bacteria
1	<i>Leminorella grimontii</i>	4 (50%)
2	<i>Enterobacter ascorbata</i>	2 (25%)
3	<i>Hafnia alvei</i>	1 (12.5%)
4	<i>Citrobacter rodentium</i>	1 (12.5%)

Table 2: Prevalence of *Bacillus* spp (n=19).

S.No.	Name of the bacteria	Percentage of the bacteria
1	<i>Bacillus amyloliquefaciens</i>	4 (21%)
2	<i>Bacillus acidicer</i>	3 (16%)
3	<i>Bacillus cereus</i>	3 (16%)
4	<i>Bacillus validus</i>	2 (11%)
5	<i>Bacillus pumilus</i>	2 (11%)
6	<i>Paenibacillus amylolyticus</i>	2 (10%)
7	<i>Bacillus stearothermophilus</i>	1 (5%)
8	<i>Bacillus sphaericus</i>	1 (5%)
9	<i>Bacillus megaterium</i>	1 (5%)

Table 3: Prevalence of fungi (n=23).

S.No.	Name of the fungi	Percentage
1	<i>Aspergillus niger</i>	10 (43%)
2	<i>Aspergillus fumigatus</i>	4(18%)
3	Yeasts	4(18%)
4	<i>Aspergillus flavus</i>	3(13%)
5	Penicillum spp	1(4%)
6	Chrysosporium spp	1(4%)

Aspergillus niger, *Aspergillus fumigatus*, Yeasts, *Aspergillus flavus*, Penicillum spp. and Chrysosporium spp. (Table 3)

Antibacterial activity of a helmet disinfectant spray

a) Spread plate method

A helmet disinfectant spray was used to assess its antibacterial activity on helmets. Two samples were collected from the helmet, one before the application of the disinfectant spray and one after the application of the disinfectant spray. The samples were vortexed and spread plated. Next day the total colonies which developed were counted with the help of colony counter and the results were recorded. The before and after results of application of a helmet disinfectant spray were observed to be 167 colonies and 91 colonies respectively.

b) Antibacterial activity of a helmet disinfectant spray by disc diffusion method

The antibacterial activity of a helmet disinfectant spray was done by disc diffusion method and it exhibited bactericidal activity by producing a zone of inhibition of 21mm.

DISCUSSION

Motorcycle helmets play a vital role in our daily life whilst the contribution of hands contaminated with

pathogenic and non-pathogenic micro-organisms leads to the spread of infectious disease. In the present study, the bacteriological analysis of motorcycle helmets showed high numbers of bacterial and fungal pathogens which was in accordance to work done by Anderson and Palombo,^[9] who found between 99-100% contaminations of dry surfaces in a research centre. Pathogens may remain infectious on surfaces for weeks after the contamination depending on environmental conditions. The longer the survival of a bacterium on a surface like the keyboard, or any other surface of an inanimate object then the potential of that pathogen being picked up by someone becomes considerably increased,^[10] Thus, the contaminated motor cycle helmets could act as vectors in transmission of pathogens. Previous works have shown that frequently used fomites are most likely to be contaminated and therefore can carry higher heterotrophic bacterial loads.^[11]

In the present study, 30 bacteria were isolated and identified. Among the 30 isolates, 3 were found to be gram positive cocci in clusters (10%) followed by 8 gram negative rods (27%) and 19 were found to be gram positive rods (63%). Among 8 g negative rods, 4 (50%) were found to be *Leminorella grimontii*, followed by 2 (25%) *Enterobacter ascorbata*, 1 (12.5%) *Hafnia alvei* and 1(12.5%) *Citrobacter rodentium*. Among 19 gram positive rods, 4 (21%) were found to be *Bacillus amyloliquefaciens*, followed by 3 (16%) *Bacillus acidicer*, 3 (16%) *Bacillus cereus*, 2 (11%) *Bacillus validus*, 2 (11%) *Bacillus pumilus*, 2 (10%) *Paenibacillus amylolyticus*, 1 (5%) *Bacillus stearothermophilus*, 1 (5%) *Bacillus sphaericus* and 1 (5%) *Bacillus megaterium*. A total of 23 fungi were isolated and identified in our study. Out of the 23 isolates, 10 (43%) were found to be *Aspergillus niger* followed by 4(18%) *Aspergillus fumigatus*, 4(18%) Yeasts, 3(13%) *Aspergillus flavus*, 1(4%) Penicillum spp and 1(4%) Chrysosporium spp.

It is important to note that most of the organisms implicated in this study are pathogenic and can lead to several health consequences.^[1] *Staphylococcus aureus* is known to cause boils, abscesses, wound infections, toxic shock syndrome, pneumonia and other disease.^[12-14] On the other hand, *Staphylococcus epidermidis* is a common skin resident responsible for endocarditis and other infections in immunocompromised patients. Its presence indicates that the use and/or sharing of motor cycle helmets can lead to transmission of serious skin infections. The presence of *Enterobacter* spp indicate possible faecal contamination of the motor cycle helmets. The consequence is that touching bike helmets might be a source of food poisoning if contaminated

hands are used in dining and preparing food without adequate hand washing cleanliness.^[13,15]

Fungal organisms identified in this study have equal health implications like the bacterial counterpart. Majority of the fungal species are widely distributed in the environment. *Aspergillus fumigatus* is ubiquitous in the environment. It is known to trigger allergic response and has been implicated in the increased incidence of severe asthma, sinusitis and pulmonary aspergillosis.^[14] Yusha'u *et al.*,^[13] found that *Aspergillus* spp. were involved in a similar study on mobile phones. *Rhizopus* spp are capable of producing toxins, which can lead to food poisoning.^[13,3]

The build-up of microbial burden within a protective motorcycle helmet occurs while it is worn. On the exterior, the helmet is hard, while from the inside, it is foamy. Following frequent usage of the helmet, this foam is susceptible to bacterial contamination and growth; this is especially true when the temperature and humidity are high (i.e. combination of factors favouring the development of micro-organisms). Therefore, these motorcycle helmets must be cleaned regularly in order to protect ourselves from being infected from the pathogenic micro-organisms. In the present study, antibacterial activity of a helmet disinfectant spray (Ustraa) was done by both spread plate method as well as by disc diffusion method. The total colonies which developed following spread plate method for both before and after the application of a helmet disinfectant spray were observed to be 167 colonies before and 91 colonies after use. The enhanced antibacterial activity of the helmet disinfectant spray may also be because of presence of isopropanol present as one of the component. The antibacterial activity of a helmet disinfectant spray by disc diffusion method exhibited bactericidal activity by producing a zone of inhibition of 21mm.

The main objective of this study was to identify the harmful effect of pathogenic bacteria by using helmets and the effect of an antibacterial spray on the micro-organism. The limitations of the study were that very less study has been done with helmets as fomites in transmission of pathogenic bacteria or use of antibacterial spray so the comparison with other research work was hard. The usage of helmet antibacterial spray may not be that prevalent in motorcycle riders as they may be unaware. This present work can give us an assurance that using such sprays can be effective against pathogenic micro-organism.

CONCLUSION

Motorcycle helmets have been identified as source of fomite which helps in the transmission of micro-organisms both bacteria and fungi which could cause severe health consequences, as per this investigation. The humid tropical weather conditions and persistent sweating while travelling by motorcyclist can also act as enhancer for the infectious diseases. We therefore recommended that effective hygiene practices can prevent the causation of microbial infection by pathogenic bacteria like *Bacillus* Species and *Aspergillus niger* which are ubiquitous in the environment. Regular cleaning of motor cycle helmets by the riders with disinfectant spray like Ustraa or any other spray can be carried out in order to reduce the incidence of microbial transmission and its associated infection. Proper cleaning regimen to sanitise these helmets regularly and public education on their hygienic usage are recommended to reduce the associated risks.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

cfu: Colony forming unit; **mm:** Millimetre; **NA:** Nutrient Agar; **MSA:** Mannitol Salt Agar; **BA:** Blood Agar; **MAC:** Mac Conkey Agar; **SDA:** Sabouraud Dextrose Agar; **MHA:** Muller Hinton Agar; **CoNS:** Coagulase Negative Staphylococci.

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