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A PILOT INVESTIGATION INTO ASSOCIATIONS BETWEEN VARIOUS INDOOR AIRBORNE BACTERIAL PARTICLES IN TRIPLICANE PUBLIC TOILET OF CHENNAI, TAMIL NADU

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ABSTRACT
The present work deals with the isolation and identification of pathogenic bacteria from an indoor toilet environment. The bacterial isolates which were isolated from an indoor public toilet in Triplicane, Chennai, Tamil Nadu, India. The identification was done on the basis of biochemical tests using selective media. The morphological characteristics of the bacteria elements showed various kinds of colony morphology, which have been identified up to genus/species level.

KEYWORDS: Gram staining, biochemical test and pathogenic bacteria.

INTRODUCTION
The analysis of microbial diversity of indoor environments is important because of its potential impact on human health. It is estimated that humans in industrialized countries spend as much as 90% of their lives indoors.[1,2] Indeed, for billions of humans, the "great indoors" comprises the new human ecosystem. Bacteria contain an enormous variety of potential microhabitats for microorganisms and are continually colonized by human and outdoor-associated microbiota.[3-5] Understanding the ecological dynamics of the microbiota in built environments may help us develop strategies to define and promote an indoor microbiome that minimizes disease risk. While it has long been known that viable bacteria can be cultured from virtually any surface in an indoor ecosystem, we know relatively little about the true diversity and viability of the indoor microbiome. In the past, studies of microbial diversity relied mainly on culture-based techniques.[6] However, the application of culture-independent sequencing techniques for the study of built environment microbiology has already greatly expanded our understanding of the origin and diversity of built environment microbes.[2]

Restrooms, are essential need for the people of any country, and it is a shared public space with clear disease transmission potential.[3] However, the potential for disease transmission from a surface fomite relies on the accumulation and continued viability of pathogenic taxa. This spatial study by Kembel *et al*. (2012)[3] revealed the dominance of human-associated microbes on restroom surfaces. In addition to that, their tolerance level and resistance level of people are different parameters that had to be taken into consideration to assess the disease dispersal. Hence, the present investigation aims to study the bacteria present in the public indoor toilet environment of Triplicane, Tamil Nadu, India.

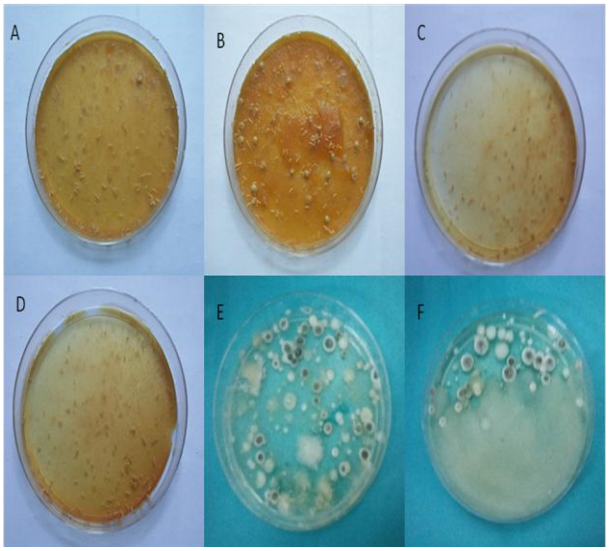
MATERIALS AND METHODS
Study area
The study was carried out in an indoor public toilet, Triplicane, Chennai, India, during 2016 - 2017.

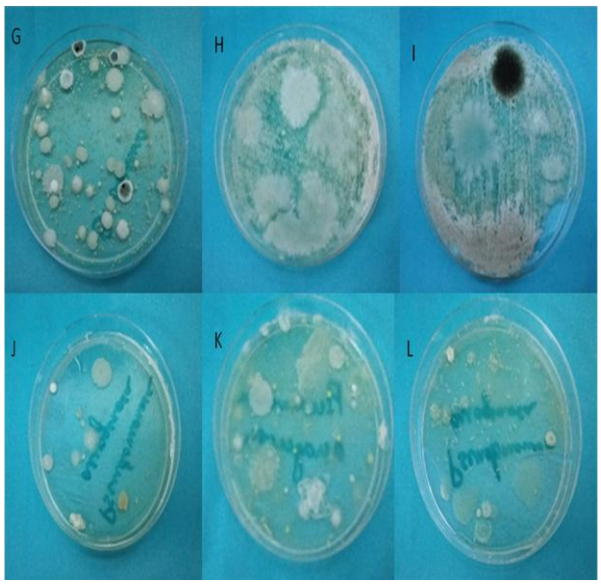
Isolation of microorganisms
Air sampling and microbial examination
The microbiological samples were collected from the indoor public toilet by exposing prepared petri dishes containing nutrient agar for the period of 48 h. Upon exposure, the plates were transported to the laboratory for examination. The bacterial culture plates were incubated at 37°C for 24 h and the bacterial colonies were initially identified by morphology and microscopic examination and identified further by biochemical tests.

Identification of bacteria
The bacteria were identified by using gram staining and biochemical analysis method of Sundararaj (1997).[7]

RESULTS
The present study investigated the airborne microbial diversity in indoor public toilet at Triplicane, Chennai, Tamil Nadu, India. The study site was exposed with Nutrient Agar (NA) for 48 h and the bacterial colony

assemblages were identified after 24 h incubation (Plates 1A-L) and the biochemical characterization of these bacterial species are depicted in Plate 2 to 9, and Table 1.





Plates 1A- L: Exposed plate showing bacterial colonies on Nutrient agar

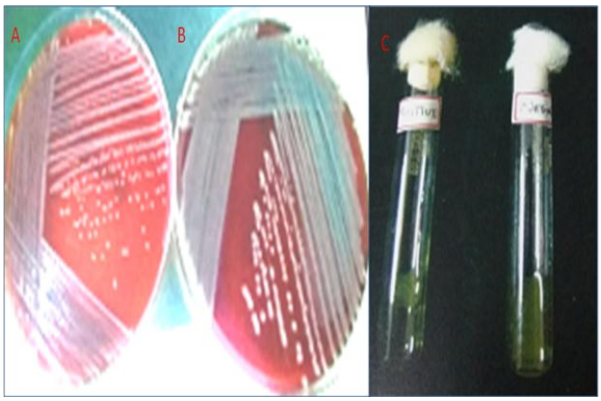


Plate 2 A – Growth of *Staphylococcus aureus* on blood agar, B - Growth of *Staphylococcus epidermidis* on blood agar, C- Tube coagulase test.

Plate 3: Growth of *Salmonella typhii* on DCA agar.

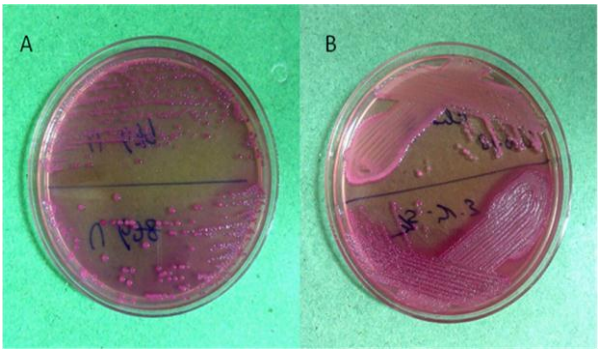


Plate 4: A- Growth of *Klebsiella pneumonia* on Mac Conkey agar B- Growth of *Escherichia coli* on Mac Conkey agar.

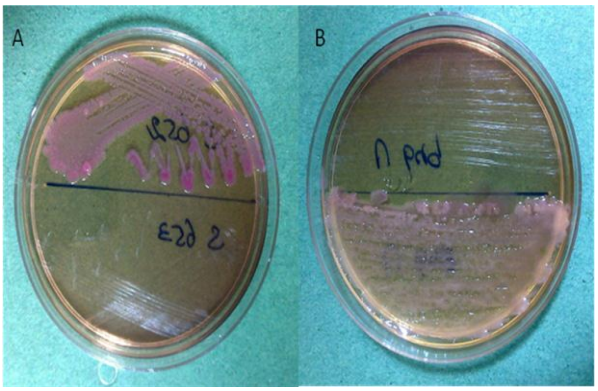


Plate 5: A- Growth of *Enterobacter cloacae* on Mac Conkey agar, B- Growth of *Proteus vulgaris* on Mac Conkey agar.

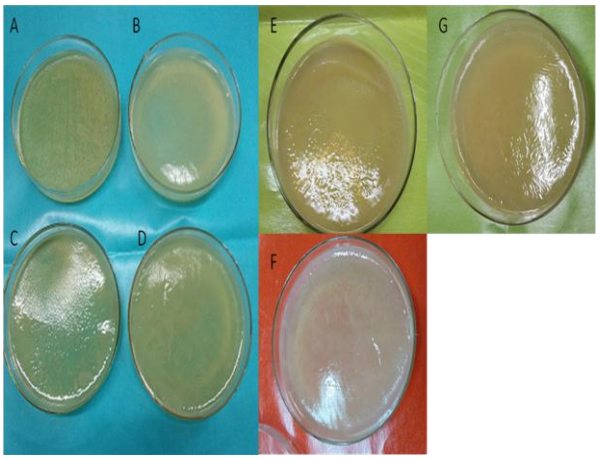


Plate 6: Growth of bacterial colonies (spread plate method) on Muller Hinton agar A- *Proteus vulgaris*,

B- *Staphylococcus aureus*, C- *Staphylococcus epidermidis*, D- *Escherichia coli*, E- *Klebsiella pneumonia*, F- *Salmonella typhi* and G- *Enterobacter cloacae.*

Plate 7 A and B: Biochemical tests for *Staphylococcus aureus* and *Escherichia coli*

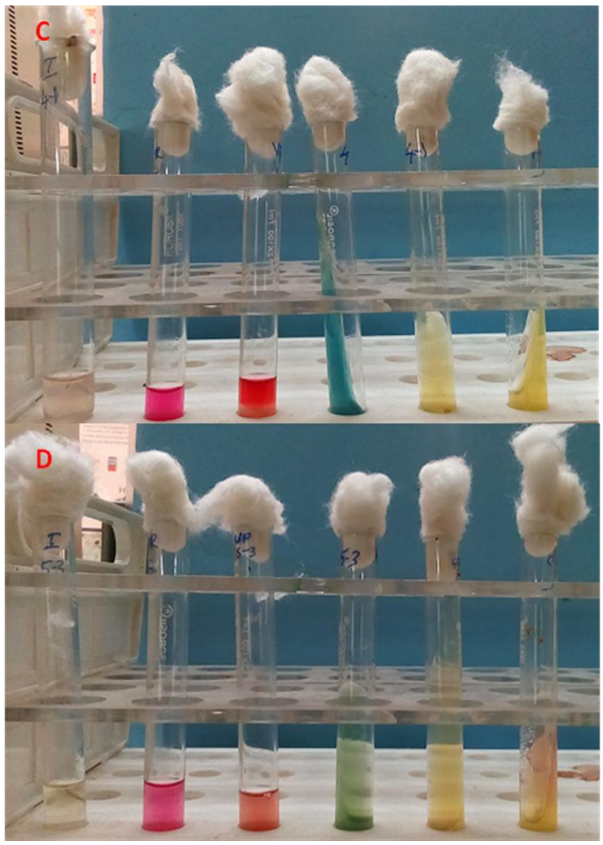


Plate 8 C and D: Biochemical tests for *Klebsiella pneumonia* and *Salmonella typhi*

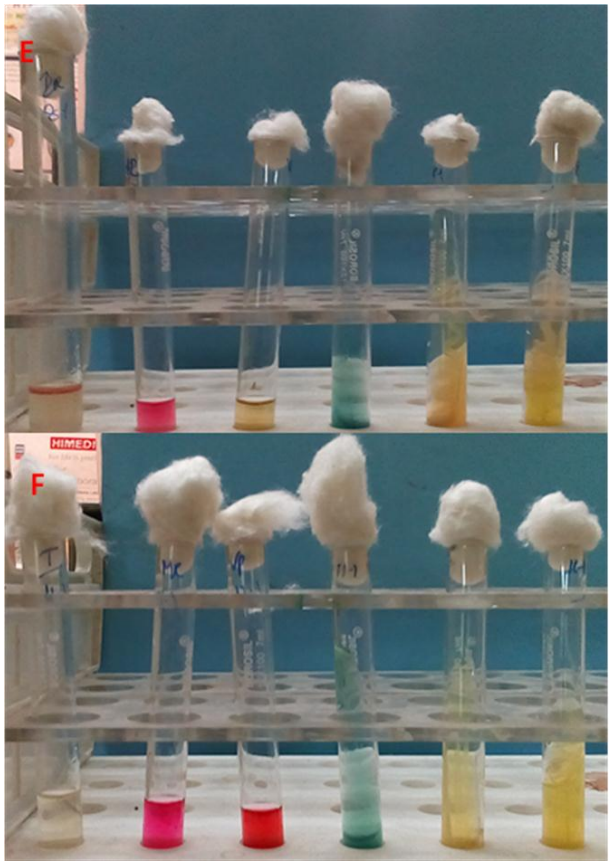


Plate 9 E and F: Biochemical tests for Proteus vulgaris and Enterobacter cloacae.

Table 1: Biochemical test of seven isolated bacterial species.

Organism	Gram staining	Motility	Catalyst	Oxidase	Cog	I	MR	VPR	CUT	TSI
S. aureus	+ ve Cocci	Non-motile	+	+	-	-	-	+	-	-
S.epidermidis	+ ve Cocci	Non-motile	+	+	-	-	-	+	-	-
E .coli	- ve Rod	Non-motile	+	-	NA	+	+	-	-	AS/G
K. pneumonia	- ve Rod	Non-motile	+	-	NA	-	+	+	+	AS/G
P. vulgaris	- ve Rod	Non-motile	-	-	NA	+	+	-	-/+	-
S.typhii	- ve Rod	Non-motile	-	-	NA	-	+	-	-	KA/ H_2S
E. cloacae	- ve Rod	Non-motile	-	-	NA	+	+	-	-	-

Whereas +: positive, -: negative, NA: not applicable, AS: acid slant/acid butt, KA: alkaline slant/acid butt, G: Gas production, H_2S: hydrogen sulphide production, I: Indole, MR: methyl red, VPR: voges prokauer test, CTU: citrate utilization test, TSI: triple sugar iron test

DISCUSSION

Generally, public rest rooms are of heavier usage and it is one of the major sources for microbial growth and contamination. As this study focused on the indoor public toilet at Triplicane, the level of contamination, threatening microbial biome at and near the place are high due to huge number of travelers. In the present investigation, the bacterial species isolated were of potential disease causing agents such as *Proteus vulgaris*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, *Klebsiella pneumonia*, *Salmonella typhi* and *Entrobacter cloacae.* Scott *et al*. (1982)[8] stated that, the public rest rooms had been greatly contaminated with microbes from human secretions as saliva, skin, urine and faecal origin. The studies of Reynolds (2005)[9] revealed that, the most implicated probable sources of infections are door handles of toilets and bathrooms. Likewise, regular cleaning of contaminated sites with different disinfectants can minimize bacterial growth; it is extremely difficult to eliminate all bacteria from surfaces.

Since air does not contain any nutrients and has low moisture content it is not conducive to the growth and survival of microorganisms but it can act as an important medium for carrying and spreading of biological agents. The order of prevalence of air microbes can vary from highest to lowest depending upon the sanitary conditions of the toilets. The reasons for high percentage of bacterial population in the toilet in the present study is due to improper and low degree of hygiene and cleanliness in the toilets. Moreover, disinfection procedures against airborne microorganisms is always less, which has given rise to bio-contaminants.

CONCLUSION AND RECOMMENDATION

Proteus vulgaris, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, *Klebsiella pneumonia*, *Salmonella typhii* and *Enterobacter cloacae* were the main bacteria isolated in this research work so far. The results of the present study demonstrated that public toilets are often colonized by several bacteria and serve as a potential source of infections. Contaminated and improperly washed toilet might result in high level of bacterial contamination, which may lead to high level prevalence of the bacterial infectious diseases. The isolation of pathogenic bacteria from the indoor public toilet indicates that they serve as vehicles of disease transmission, as microbial contamination of toilet indoor surfaces may be a common means of transfer of potentially pathogenic bacteria among users. Based on the above findings, it is therefore recommended that the Chennai Corporation should insist the regular usage of sanitizers to the users or spray disinfectants with regular cleaning of the toilets to ensure reduction in the microbial load. More number janitors should be employed especially in public toilets located in heavily crowded places. Individuals both adult and young should adopt the habit of hand washing practice after using the toilets and routine surface disinfection of the toilets door handles and floors, which can prevent cross contamination. Besides encouraging for general good hygiene practice by all, there is a need for the Chennai Corporation to provide more toilets and increase its sanitation frequencies.

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