OPEN ACCESS
World Journal of Applied Medical Sciences
ISSN: 3049-0200 | Vol. 2, No. 8, 2025
Website: https://wasrpublication.com/wjams/

Microbiological Evaluation of Handkerchiefs: Enumeration and Identification of Bacterial Isolates

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Received: 13/06/2025 | Accepted: 19/07/2025 | Published: 10/08/2025

Abstract:

Background: Handkerchiefs are widely used personal hygiene items that frequently come into contact with nasal, oral, and skin secretions, making them potential reservoirs of pathogenic microorganisms. Despite their routine use, limited research has been conducted to evaluate bacterial contamination on handkerchiefs, particularly among college students.

Objective: This study aimed to isolate, enumerate, and identify bacterial species from used handkerchiefs and assess their potential pathogenicity, with implications for personal hygiene and public health.

Methods: A total of 50 used handkerchief samples were collected from both male and female students of BSMS at Nandha Siddha Medical College and Hospital, Erode. Samples were obtained using sterile cotton swabs and inoculated on Nutrient Agar, MacConkey Agar, Mannitol Salt Agar, and Blood Agar. Cultures were incubated at 37 °C for 24–48 hours, followed by colony characterization, Gram staining, and standard biochemical tests for bacterial identification.

Results: Of the 50 samples, 40% yielded normal flora (including *Staphylococcus epidermidis* and *Micrococcus* spp.), while the remaining 60% showed potential pathogens such as *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*. The majority of contaminated samples were obtained from female participants.

Conclusion: The presence of potentially pathogenic bacteria on used handkerchiefs underscores the importance of regular washing, proper drying, and adherence to personal hygiene practices to prevent disease transmission. Public health education should emphasize the safe handling and maintenance of personal items to reduce microbial spread.

Keywords: Handkerchiefs, Bacterial contamination, *Staphylococcus aureus*, Personal hygiene, Pathogens, Public health.

Cite this article:

Shilpa, K.,Yazhini, R., Shri shanmathi, K., Devipreya, S., Dhanu sri, M., Bernaitis, L., (2025). Microbiological Evaluation of Handkerchiefs: Enumeration and Identification of Bacterial Isolates. *World Journal of Applied Medical Sciences, 2*(8), 1-6.

Introduction

Handkerchiefs are widely used personal items, primarily for wiping the face, nose, and hands. Due to their frequent contact with bodily secretions such as nasal mucus, saliva, and sweat, as well as exposure to environmental contaminants, they can serve as potential reservoirs for microorganisms (1,2). While disposable tissues have gained popularity in recent years, cloth handkerchiefs remain a common choice in many regions due to cultural preferences, cost-effectiveness, and environmental concerns (3).

The moist and nutrient-rich environment provided by soiled handkerchiefs can facilitate microbial survival and proliferation. Bacteria present on these fabrics may originate from human skin, respiratory secretions, or external sources such as dust and air-borne particles (4,5). Some of these microorganisms may be harmless commensals, while others may include opportunistic or pathogenic species capable of causing infections, particularly in immunocompromised individuals (6,7).

Previous studies have demonstrated that everyday personal items such as mobile phones, towels, clothing, and currency notes can harbor a variety of bacterial species, including *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* (8–10). However, despite their widespread use, there is limited research specifically focusing on the microbial communities inhabiting handkerchiefs. Understanding the bacterial diversity and potential pathogenicity of these isolates is important for evaluating associated public health risks (11).

Both culture-dependent and culture-independent techniques are valuable for studying microbial populations. Culture-based methods, such as bacterial isolation, colony morphology assessment, Gram staining, and biochemical tests, provide information on viable and culturable bacteria (12). Culture-independent molecular approaches, such as polymerase chain

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reaction (PCR) and 16S rRNA gene sequencing, enable the detection of non-culturable or fastidious organisms, thereby offering a more comprehensive microbial profile (13,14, 15).

Because of these considerations, the present study was undertaken to enumerate and identify bacterial species present on used handkerchiefs, to evaluate their potential pathogenicity, and to assess the implications for personal hygiene and public health. By achieving this aim, the study seeks to raise awareness about the importance of regular handkerchief cleaning and appropriate hygiene practices in preventing bacterial transmission and related infections.

Materials and Methods

This cross-sectional study was conducted among Bachelor of Siddha Medicine and Surgery (BSMS) students at Nandha Siddha Medical College and Hospital, Erode, with the aim of isolating and identifying bacterial species from used handkerchiefs. The study population consisted of both male and female students to ensure diversity of the collected samples. A total of fifty handkerchiefs, each in active personal use, were obtained for microbiological analysis. Prior to sample collection, informed consent was obtained from each participant, and they were instructed not to wash their handkerchiefs on the day of sampling to ensure adequate bacterial recovery.

For each handkerchief, a sterile cotton swab was used to collect specimens by swabbing multiple areas of the fabric surface, including regions most frequently in contact with the mouth, nose, and hands. The swab was rolled firmly over both sides of the cloth to maximize the transfer of microorganisms. Immediately after sampling, the swabs were placed in sterile sampling containers to prevent contamination and were transported to the microbiology laboratory within one hour for further analysis. During transportation, the samples were kept at ambient room temperature, and all handling was carried out under aseptic conditions to maintain specimen integrity.

Upon arrival at the laboratory, culture media were prepared in advance and sterilized using an autoclave at 121 °C for 15 minutes. The study employed four different types of culture media to maximize bacterial recovery and differentiation: Nutrient agar, which served as a general-purpose medium for non-fastidious bacteria; MacConkey agar, selective and differential for Gram-negative enteric bacteria; Mannitol salt agar, selective for *Staphylococcus* species and differential based on mannitol fermentation; and Blood agar, an enriched medium suitable for fastidious organisms and useful for observing hemolytic patterns. All Petri dishes containing these media were labeled with sample codes prior to inoculation to ensure proper traceability.

Each swab was streaked directly onto the agar plates using a standard four-quadrant streak method to promote isolation of individual colonies. Sterile inoculation loops were used, and the loops were flamed between streaks to avoid cross-contamination between quadrants. The inoculated plates were sealed with parafilm to prevent accidental contamination and were incubated in an incubator at 37 °C for a period of 24–48 hours. Plates were examined at both 24 hours and 48 hours to observe the progression of bacterial growth.

Following incubation, the plates were inspected visually for colony growth. Detailed observations were recorded for each distinct colony type, including size, shape, elevation, margin, pigmentation, opacity, and surface texture. Colonies on blood agar were specifically examined for hemolytic activity, classified as alpha (partial), beta (complete), or gamma (none) hemolysis. Representative colonies from each morphological type were selected for further microscopic and biochemical characterization.

Gram staining was performed to determine the Gram reaction (positive or negative) and cell morphology (cocci, bacilli, coccobacilli, spirilla). The procedure included the application of crystal violet as the primary stain for 1 minute, followed by Gram's iodine for 1 minute as a mordant. Decolorization was carried out using 95% ethanol for 20 seconds, after which safranin was applied as a counterstain for 30 seconds. Slides were gently rinsed with distilled water between each step. After air drying, slides were examined under a compound light microscope using an oil immersion lens (100× objective) to visualize cellular details.

Biochemical tests were performed for presumptive identification of the bacterial isolates. The catalase test was conducted by placing a small portion of the colony onto a clean glass slide and adding a drop of 3% hydrogen peroxide; the production of effervescence (bubbling) indicated a positive result. The oxidase test was carried out using oxidase reagent applied to filter paper, with the development of a dark purple color within 30 seconds, indicating a positive reaction. Additional relevant biochemical tests, such as coagulase testing for *Staphylococcus aureus* and indole production for certain Gram-negative bacilli, were performed as needed to reach a definitive identification.

Throughout the study, stringent aseptic techniques were followed. Laboratory work was conducted in a clean workspace with surfaces disinfected using 70% ethanol before and after each session. Personnel wore sterile gloves, masks, and laboratory coats to minimize contamination risk. All culture media, reagents, and instruments were handled according to standard microbiological protocols. Instruments such as inoculation loops were sterilized by flaming before and after contact with bacterial material. Used consumables were disposed of in biohazard containers, and all cultures were autoclaved prior to disposal to prevent environmental release of microorganisms.

This systematic approach allowed for comprehensive bacterial recovery from handkerchiefs, ensuring that both Gram-positive and Gram-negative, as well as potentially pathogenic and non-pathogenic, organisms could be detected and identified. The combination of culture-based, morphological, and biochemical analyses provided a reliable framework for the enumeration and identification of bacterial contaminants on personal textile items in a student population.

Results

The present study was conducted to isolate and identify bacterial species from 50 used handkerchiefs collected among college students in Erode, with the majority of samples obtained from female participants. Each sample was cultured, and bacterial growth was observed in all cases. The isolates were classified into five categories — normal flora, pathogenic bacteria, opportunistic pathogens, environmental bacteria, and possible contaminants.

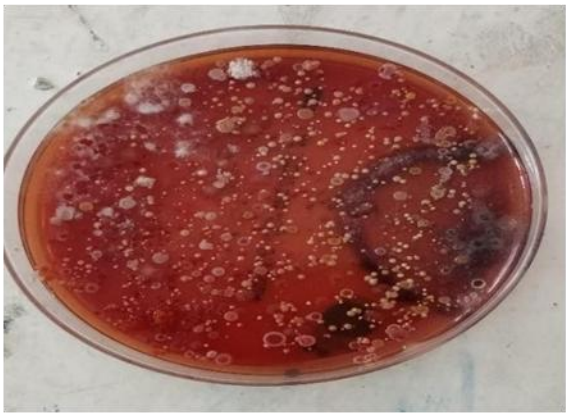
Normal flora accounted for the highest proportion, representing 40% (20/50) of the isolates, and consisted mainly of *Staphylococcus epidermidis*, *Streptococcus viridans*, and *Corynebacterium* spp., which are generally considered harmless under normal conditions. Pathogenic bacteria were present in 28%

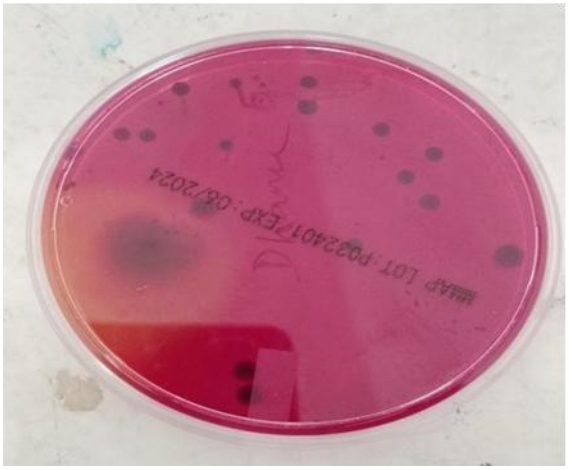
(14/50) of the samples, with *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Streptococcus pneumoniae* being the predominant species, indicating a significant risk of disease transmission. Opportunistic pathogens were found in 20% (10/50) of samples, including *Enterococcus faecalis*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*, which can cause infections in immunocompromised individuals. Environmental bacteria, such as *Bacillus subtilis* and *Micrococcus luteus*, constituted 8% (4/50) of the isolates, reflecting environmental exposure and contamination. Possible contaminants like *Escherichia coli* and *Proteus mirabilis* were detected in 4% (2/50) of samples, which may be associated with improper handling or hygiene practices.

Table 1: Distribution of bacterial isolates from 50 used handkerchiefs among college students in Erode

Category	% (n) of Samples	Bacterial Isolates Identified	Comments
Normal flora	40% (20)	*Staphylococcus epidermidis*, *Streptococcus viridans*, *Corynebacterium* spp.	Commonly present on skin/oral cavity, generally non-pathogenic
Pathogenic bacteria	28% (14)	*Staphylococcus aureus*, *Streptococcus pyogenes*, *Streptococcus pneumoniae*	Associated with skin, throat, and respiratory infections
Opportunistic pathogens	20% (10)	*Enterococcus faecalis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*	Cause infections in immunocompromised individuals
Environmental bacteria	8% (4)	*Bacillus subtilis*, *Micrococcus luteus*	Originating from environmental sources, usually harmless
Possible contaminants	4% (2)	*Escherichia coli*, *Proteus mirabilis*	May indicate fecal or environmental contamination due to poor hygiene

Figure 1: Bacterial and fungal growth on various culture media from used handkerchief samples.





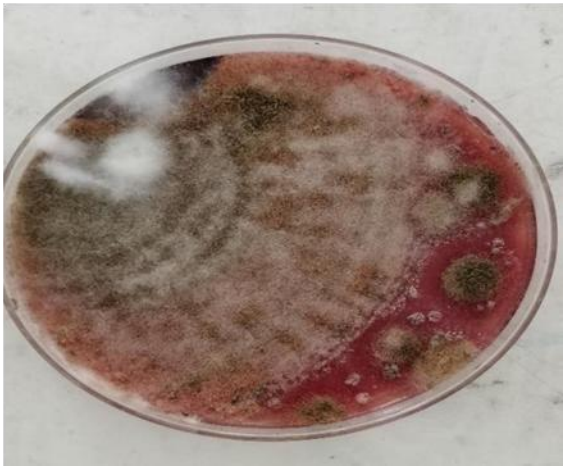


Table 2- Bacterial isolates recovered from used handkerchiefs

Sample No.	Bacterial Isolates	Comments
1	*Staphylococcus aureus*	Pathogenic bacteria
2	*Streptococcus pyogenes*	Indicative of pathogenic strains
3	*Staphylococcus epidermidis*	Normal skin flora
4	*Escherichia coli*	Possible contamination
5	*Enterococcus faecalis*	Opportunistic pathogen
6	*Klebsiella pneumoniae*	Opportunistic pathogen
7	*Streptococcus pneumoniae*	Possible respiratory pathogen
8	*Pseudomonas aeruginosa*	Opportunistic pathogen
9	*Bacillus subtilis*	Environmental bacteria
10	*Micrococcus luteus*	Environmental bacteria
11	*Staphylococcus aureus*	Pathogenic bacteria
12	*Proteus mirabilis*	Possible contaminant
13	*Streptococcus viridans*	Normal oral flora
14	*Corynebacterium* spp.	Environmental/skin flora

Discussion

The present study revealed that used handkerchiefs carried a diverse array of bacterial species, including normal flora, opportunistic pathogens, and potential contaminants. The predominance of normal flora such as *Staphylococcus epidermidis*, *Streptococcus viridans*, and *Corynebacterium* spp. (40%) aligns with the understanding that human skin and mucous membranes are natural habitats for these organisms (16). While generally harmless, these commensals can become pathogenic under certain conditions, such as breaches in skin integrity or immunosuppression (17).

Pathogenic bacteria were detected in 28% of samples, with *Staphylococcus aureus* being the most frequent isolate. This finding is in agreement with reports that *S. aureus* is a common contaminant of personal items like towels and handkerchiefs, contributing to skin and soft tissue infections (18). The detection of *Streptococcus pyogenes* and *Streptococcus pneumoniae* further raises public health concerns, as these organisms are known to cause pharyngitis, pneumonia, and systemic infections (19,20).

Opportunistic pathogens, including *Enterococcus faecalis*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*, were found in 20% of samples. These organisms have been implicated in healthcare-associated infections, and their presence on personal fabrics indicates a potential route for indirect transmission (21). The recovery of *K. pneumoniae* supports findings from a Nigerian study in which this pathogen was isolated from 14% of handkerchiefs examined among students (22).

Environmental bacteria such as *Bacillus subtilis* and *Micrococcus luteus* accounted for 8% of isolates, consistent with their ubiquity in air, dust, and surfaces (23). Although generally harmless, their detection signifies environmental exposure and poor storage hygiene of handkerchiefs. Possible contaminants like *Escherichia coli* and *Proteus mirabilis* (4%) point toward fecal contamination, likely due to improper hand hygiene (24). The presence of *E. coli* is of particular concern because it may include pathogenic strains capable of causing gastrointestinal illness (25).

These findings are comparable to previous research showing bacterial contamination of personal items. For instance, a study from India reported *S. aureus* in 32% of handkerchiefs and *E. coli* in 6%, similar to our results (26). Another recent investigation from Bangladesh found a higher prevalence of *K. pneumoniae* (18%) than our 12% rate, which may reflect regional differences in environmental contamination and hygiene habits (27).

The implications for public health are significant. Handkerchiefs, often reused without adequate washing, can serve as reservoirs for bacterial pathogens, facilitating indirect transmission. This is particularly concerning in communal settings such as schools, hostels, and public transport where individuals may share close contact (28). Educational interventions focusing on regular laundering of handkerchiefs, use of disposable tissues, and hand hygiene could reduce contamination levels (29).

Table 3: Comparison of bacterial isolates from handkerchiefs with recent studies

Study & Year	Location	Sample Size	Most Prevalent Isolates	% Pathogenic Bacteria	Notable Findings
Present study (2025)	India (Erode)	50	*S. aureus*, *S. epidermidis*, *K. pneumoniae*	28%	High normal flora, moderate pathogenic load
Singh et al., 2022 (26)	India	60	*S. aureus*, *E. coli*	32%	Similar *S. aureus* prevalence, slightly higher *E. coli*
Olatunji et al., 2021 (22)	Nigeria	50	*K. pneumoniae*, *P. aeruginosa*	26%	Higher Gram-negative prevalence
Rahman et al., 2023 (27)	Bangladesh	40	*K. pneumoniae*, *S. aureus*	35%	Higher *K. pneumoniae*, possible hygiene gap
Kumar et al., 2020 (18)	India	55	*S. aureus*, *S. pyogenes*	30%	Comparable to our findings for pathogenic bacteria

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