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Isolation and identification of bacterial contamination from used pens by using nutrient agar medium

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Abstract

The pen is a common writing instrument made of plastic or metal with a pointed tip used to apply ink to a surface. Placing pen on dirty surfaces like other objects exposes it to contamination. In this study was conducted to isolate and identify the bacterial contamination of student's pen in Sathyam International School, vellakoil, Tiruppur, Tamil Nadu, India by using nutrient agar medium. Samples were prepared using swabs moistened with sterile saline and streaked on nutrient agar plates. Out of 25 samples, 19 samples are contaminated by bacteria such as Escherichia coli and Staphylococcus aureus. Escherichia coli having the highest amount and followed by Staphylococcus aureus having lowest amount colony forming capacity.

Keywords: Pen; Bacteria; Contamination; Nutrient; Pathogen

1. Introduction

The Bacteria are tiny, single-celled living organisms. There are millions of different types of bacteria, found almost everywhere on Earth and are vital to the planet's ecosystems. Many can be found in and on your body and are beneficial to you [1]. These bacteria make up your microbiome, which keeps your body healthy. Other bacteria can make you sick. These bacteria are a type of pathogen. Pathogens are microorganisms that can cause disease. They can reproduce quickly in your body and give off poisons (toxins) that can cause infection. Bacteria continue to grow on them because of their wet and moist surface [2].

The contamination of objects could be from several sources like atmosphere, during storage, handling, or production [3]. The microorganisms that are mostly isolated from surfaces of objects included members of the genus *Staphylococcus* species, *Enterobacteria*, and *Bacilli* sp. Most objects contaminants are environmental microorganisms and those arising from human skin flora such as *Staphylococcus aureus* [4]. The presence of bacteria on objects reflects the local environmental situation and personal hygiene.

The pen is a common writing instrument made of plastic or metal with a pointed tip used to apply ink to a surface, usually paper, for writing or drawing [5]. A pen as an object that is passed from hand to hand is likely to be contaminated with diseases causing microorganisms especially if handled with unclean hands or kept in dirty surroundings [6]. Pen, therefore, presents a particular risk to public health since communicable diseases spread through contact with fomites [7]. The source of contaminations and infections could be because of poor or negative handling practices like chewing contaminated objects in the mouth or scratching of body parts with their edge or head [8]. Placing pen on dirty surfaces like other objects exposes it to contamination, making it a convenient habitat for pathogens [8].

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In this study was conducted to isolate and identify the bacterial contamination of student's pen in Sathyam International School, Vellakoil, Tiruppur, Tamil Nadu, India by using nutrient agar medium.

2. Material and methods

2.1. Collection of Sample

25 samples were obtained randomly during July 2023, from the students of Sathyam International School, Vellakoil, Tiruppur, Tamil Nadu, India, on co-ordinates 10°88'3068"N, 77°72'2976"E elevation of 192 metre.

2.2. Preparation of Media

Suspend 28 g of nutrient agar powder in 1 litre of distilled water. Heat this mixture while stirring to fully dissolve all components. Autoclave the dissolved mixture at 121˚C for 15 minutes. Once the nutrient agar has been autoclaved, allow it to cool but not solidify. Pour nutrient agar into each plate and leave plates on the sterile surface until the agar has solidified. Replace the lid of each Petri dish and store the plates in a refrigerator.

2.3. Sample preparation and Inoculation

Samples were prepared using swabs moistened with sterile saline and streaked on nutrient agar plates. All the plates were incubated at 37 °C for 24 hours and observed for growth. The colonies were counted using colony counter.

2.4. Identification of isolated bacteria

After isolating pure cultures, the bacterial isolates were then identified by observing their growth on potato agar for the morphological characteristic. Gram staining reaction and biochemical tests such as catalase test, coagulate test and indole test were used to confirm the isolated bacteria as described by Cheesbrough [9, 10].

3. Results and discussion

Out of 25 sample 19 samples are contaminated by bacteria such as *Escherichia coli* and *Staphylococcus aureus. Escherichia coli* having the highest amount and followed by *Staphylococcus aureus* having lowest amount colony forming capacity. The distribution of bacteria is shown in figure 1. The frequency of isolated bacteria is *Escherichia coli* and *Staphylococcus aureus.* 63% and 37% respectively.

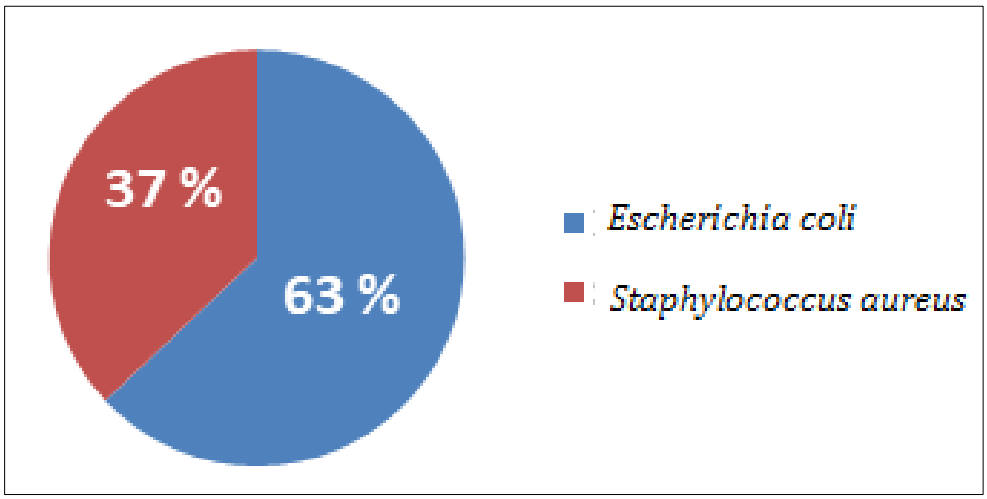

Figure 1 Distribution of bacterial colonies in the present study

These bacteria may probably have found their entry to the pens through the skin and hand to hand mechanism since they are normal microbiota of the skin as advanced by Pope et al. [7] his is a cause for concern since the greater the concentration of bacteria on the object, the longer their ability to survive and these pathogenic bacteria may cause disease in anyone who gets contaminated while using the object according to Reynolds et al. [11]

Table 1 Characterization of bacterial colonies isolated from the pen.

Characteristics of culture	**Gram staining**	**Shape of the bacteria**	**Arrangement of colonies**	**Conformation Tests**				**Suggested organism**
				Catalase	Coagulase	Indole	Mortality	
Round, smooth, raised,	Positive	Cocci	Clusters	Positive	Positive	Negative	Negative	*Staphylococcus aureus*
Circular, smooth colonies with distinct edges	Negative	Bacilli	Singly	Negative	Negative	Positive	Positive	*Escherichia coli*

4. Conclusion

Bacteria are impossible to avoid, but knowing where to find them makes it easier to keep your items clean and avoid the spread of bacteria or contamination. This study has shown that pen can be contaminated by bacteria and could serve as a potential source of infection to transmit diseases from one person to another. Therefore, it is important to advise hand washing, personal hygienic practice, and routine surface disinfection of personal items, especially among the students to prevent the spread of bacterial infections.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

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