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Common lab contaminants responsible for spoilage in a pharmaceutical college laboratory

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Abstract
Numerous items have been shown to spoil in laboratories due to the presence of microbial contamination. Chemical and pharmaceutical working solutions are particularly susceptible to harm and contamination from indoor pollution. For reasons of safety as well as the validity of tests and experiments that must be conducted in non-contaminated surroundings, an assessment of the microbiological contamination of such materials may be of utmost significance. In this study, an effort was made to look at the bacterial flora in the lab waste from the pharmacy college and identify some of the isolates partially utilizing cultural, microscopic, and biochemical features. Before entering test rooms, recreation rooms, canteens, libraries, or any other area of the building, protective clothes worn in the lab must be removed. It's a good idea to wash your hands with disinfectant before and after conducting any experiment. We can infer from our experiment that laboratory reagents over time harbor a large number of microorganisms. Therefore, we should take preventative precautions like quality inspecting labs and inhibiting bacteria to ensure error-free tests.

Keywords: Laboratory, lab contaminants, biochemical tests, bacteria, staining, bacteria culture

Introduction
Presence of microbial contaminants in laboratories is found to cause spoilage of numerous products. Working solutions of Chemicals and pharmaceutical products are most vulnerable to damage and exposure to contamination by indoor pollutants. An evaluation of the microbial contamination of such materials could be of crucial importance, both for safety reasons and for the reliability of tests and experiments that need to be carried out in non-contaminated environments. Contamination is undesired introduction of impurities like chemical, microbial or physical matter, into or onto a product of any laboratory during sampling, holding, processing, storing, transferring, packaging and transporting (Abatenh *et al*., 2018) [1]. Physical contamination occurs by entry of physical material like fibers, dust etc. Chemical changes occur by molecular degradation due to physical parameters. The most important problem lies with biological spoilage which occurs by entry of microbes. Microbial contamination is very common and fast as microbes are ubiquitous in nature. Ultimately the contamination leads to damage or spoilage of the particular product.
Microbial contamination is one of a biggest worldwide obstacle for laboratory work. It is a global concern regarding health and leads to difficult for getting accurate research output. It is manually or systematically introduced in our experiments and damages the quality of our work (Borst *et al*., 2004) [2].
Several researchers reported about presence of contaminants in common products and reagents stored in a laboratory such as (Kosif and Avcioglu (2018) [3], Isola and Olatunji (2016), Ghayoor *et al*., (2015) [4, 5].
In this work, an effort was undertaken to examine the bacterial flora in the laboratory waste from the pharmacy college and to identify some of the isolates partially using cultural, microscopic, and biochemical traits.

Methodology
1. Collection of samples
Different pharmaceutical products which were seemed to be spoiled and being kept in the Laboratories since long for experimental purposes were taken as samples for the present study to observe the microbial contaminants behind the spoilage.

2. Isolation of bacterial strains as contaminants
For this purpose Nutrient Agar plate was prepared according to the manufacturer's instruction. Then the media was autoclaved at 1210C/15 lbs for 15 min. After solidification of the media, spread plate method was followed for isolation. 100 µl from each sample was transferred to the NA surface. It was then spread over the agar surface. The plates were incubated at 370C for a period of 24 hrs. The appearances of growth in the form of colonies were taken into account for further studies.

3. Bacteriological analysis of the contaminants
After the isolation of bacterial strains on the Nutrient agar plates, the observation was taken visually with appearance of bacterial colonies on the surface of NA plates. The colonies were observed and grouped according to their distinguishing characteristics shown on the nutrient plate. One isolated colony from each group was taken and streaked on fresh Nutrient Agar plate to get the pure culture. Then it was incubated at 370 ℃ for 24 hours. After this, the cultures were identified on the basis of their morphological, microscopic and biochemical characteristics.

4. Cultural characteristics study
It is the characterization of colony by direct visualization. The isolated strain grown on NA plates was subjected to identification by following diagnostic microbiology based on the standard physical parameters like Colony appearance, shape, margin, elevation, texture, optical density, coloration, odor etc.

5. Microscopic study
The contaminants were subjected to microscopic study. The isolated colonies were Gram stained (using Crystal Violet as primary staining reagent, Iodine as mordant, Safranin as counter staining reagent and absolute alcohol ad decolorizing agent) and the cells were visualized under the optical microscope at various magnifications (100X, 400X and 1000X).

6. Biochemical characterization
The following biochemical tests were performed to partially identify the contaminants.

6.1. Indole test
Sterile peptone water was taken in test tubes and the overnight cultures of the isolates were inoculated. After incubating at 370 C for 48 hrs, Kovac's Indole reagent was added to the growing culture broth slowly. The tubes were observed for color change after a mild shaking. For the sake of confirmation, the test tubes were kept for 2 hrs.

6.2. Methyl red test
The test organism were inoculated to 1% Glucose broth and incubated at 370 C for 48 hrs. Then Methyl Red indicator was added to the culture broth and the same was observed for any colour change. Napthol) and 0.6 mlα

6.3. Voges-proskauer test
0.6 ml of Voges-Proskauer reagent A (of Voges-Proskauer Reagent B (40% KOH) were added to a growing culture broth (in 1% Glucose) after 48 hrs. of incubation at 370 C. The tubes were kept to stand for 2 hrs. To observe the reaction indicated by color change.

6.4. Citrate utilization test
The isolates were grown on Simmon's Citrate Agar slants for 48 hrs. and the slants were observed whether the media retained its green color or changed to blue.

Results
1. Isolation of bacterial strains as contaminants
Mixed cultures of bacterial colonies were obtained from the laboratory reagent samples (Fig # 1). From the mixed culture, five types of colonies were selected over the agar plate for further study basing upon the distinguished morphology *viz*. C1, C2, C3, C4, and C5. They were subjected for further identification and assays.

2. Cultural characteristics
The results regarding the colony characteristics obtained from the streaking of the five contaminant bacteria on NA plates are displayed in Table #1 and Fig # 2. From the mother culture plates it was observed that the colonies were separate and distinct. They were then sub cultured and preserved for additional assays.

3. Microscopic study
The response showed by the isolates towards the treatment of Grams reagents is depicted in Fig # 3 and Table # 2.

4. Biochemical test
The observations of biochemical response (IMViC) of the five lab contaminants were depicted in Table # 3 and Fig # 4. Regarding the Biochemical characterization, all the contaminants were found to be Indole negative and also Voges-Proskauer negative. Two strains showed negative to Methyl Red while three were positive. Those isolates were able to degrade Glucose with production of acid which changes the colour of the medium to Red after adding the indicator Methyl Red. All the strains could utilize Citrate which was confirmed from the colour change of the medium from forest green to blue and all others were negative for Citrate Utilization.

Table 1: Cultural characteristics of the five contaminants

Isolate	Morphology
C1	Rough, large size, circular, flat, irregular margin, undulate, yellowish white colour, semi transparent
C2	Smooth, shiny, medium size, circular, convex, entire margin, Orange, opaque
C3	Smooth, small, circular, flat, entire margin, lemon yellow colour, opaque
C4	Smooth, shiny, large size, circular, convex, entire margin, white colour, semi transparent
C5	Smooth, shiny, medium size, circular, raised, entire margin, White colour, transparent

Table 2: Grams Response

Isolate	Gram's Response	Shape
C1	+	Coccus
C2	+	Coccus
C3	-	Coccus
C4	-	Rod
C5	+	Coccus

Table 3: Response of the contaminants to Biochemical reactions

Isolate	Indole	Methyl Red	Voges Proskauer	Citrate Utilization
C1	-	+	-	+
C2	-	+	-	+
C3	-	-	-	+
C4	-	-	-	+
C5	-	+	-	+

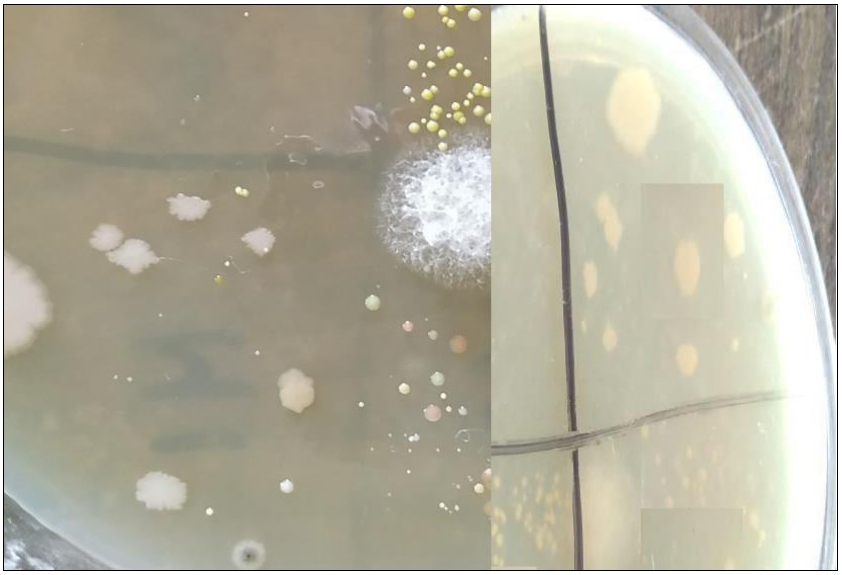

Fig 1: Isolation plate showing mixed bacterial isolates

Fig 2: showing the cultural growth of the five contaminants

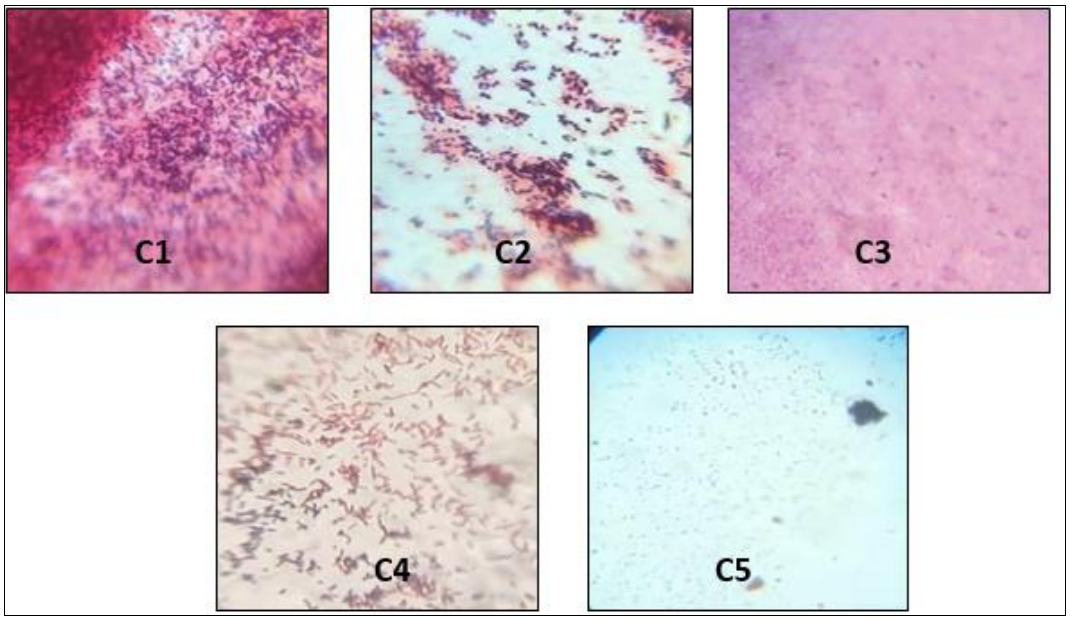

Fig 3: Microscopic view of the isolates under 1000X

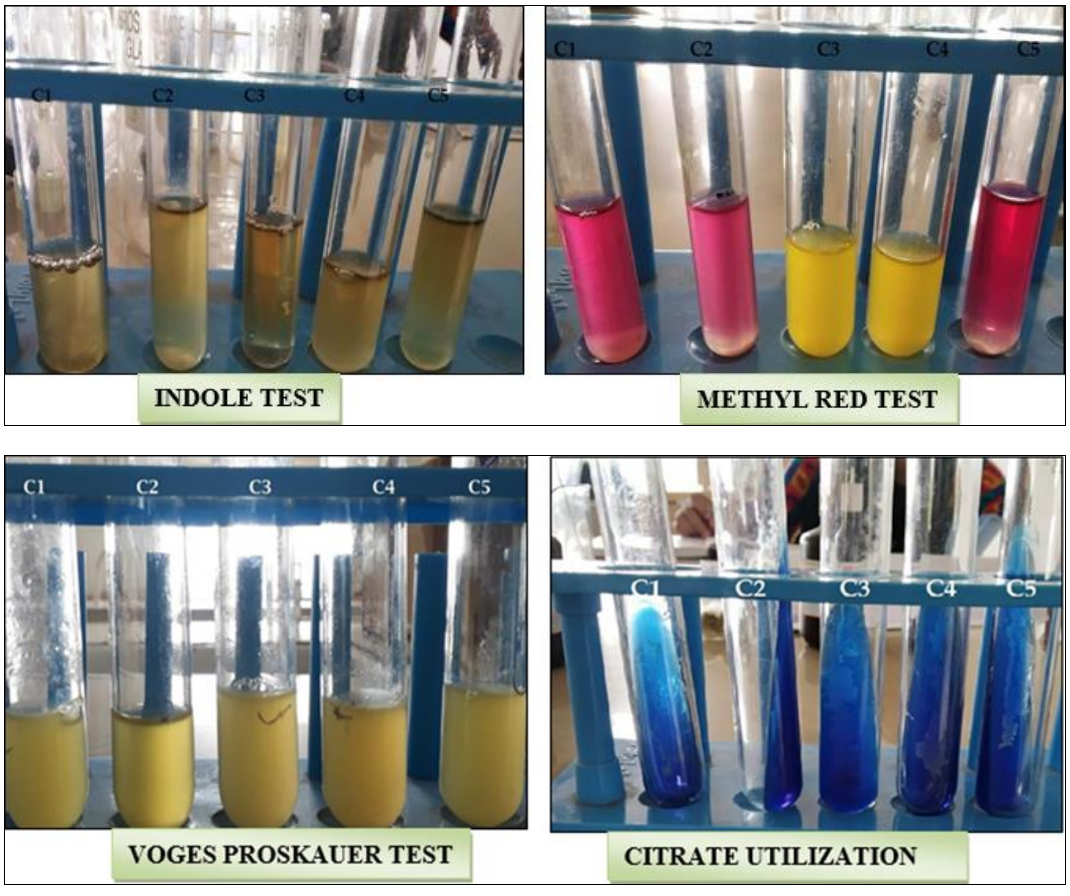

Fig 4: Biochemical reaction of the isolates

Conclusion

Laboratory is contaminated through many means such as air, animal, or human agents when talking, sneezing, and coughing in the laboratory. There is a possibility of transferring microorganisms from one laboratory to the other during the course of activities that involves moving of materials. Control measures that are adequate for the routine circumstances of daily life might be used such as cleaning with soaps and detergents to control undesirable microorganism to some extent. When working in the laboratory, all staff and students must wear protective clothing e.g. lab coat which should not be worn for more than two days and should be placed in receptacle prior to autoclaving. Protective clothing worn in the laboratory must be taken off before visiting test rooms, recreational rooms, canteens, library or other part of the premises. Washing hands in disinfectant before and after any experiment is a good practice. From our experiment it may be concluded that the laboratory reagents harbour many microbes during the course of time. Hence for error free experiments we

should take precautionary measures by quality checking of labs and inhibition of microbes.

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