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**Isolation and Identification of Common Contaminants Bacteria from
Working Area in Microbiology Laboratory**

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Abstract: Frequent environmental contaminants within microbiology laboratory create not only diagnostic quandaries but also poses major risk for health care workers and patients. Objective of our study was to isolate and identify the common laboratory contaminant bacteria with an ultimate goal to reduce false positive culture reports as well as Laboratory acquired infections. The study was conducted in laboratory of Microbiology Department Kohat University of Science and Technology Kohat from March 2014 to June 2014. Total 5 samples were collected from different areas of laboratory including table, floor, clothing, air, and incubator. Out of 5 collected samples, growth was observed. Out of 5 culture positive samples total 22 bacterial contaminants were isolated and identified by various biochemical tests. Out of 22 bacterial contaminants, 8 (36.36%) was *Staphylococcus epidermis*'s which was the most common contaminants. 7 (31.81%) was *Bacillus subtilis* which was the second most common contaminants. 4 (18.18%) was *Staphylococcus aerous* while 3 (13.63%) was *Depthierods* which showed minimum bacterial contaminants. Precaution should be taken to get rid of these organisms from laboratory by means of proper laboratory disinfection and sterilization as well as personal hygiene of laboratory workers. From our study we conclude that laboratory can be a potential source of contamination to give false positive results.

Key words: Laboratory contaminant, *Staphylococcus epidermises*, *Bacillus subtilis*

Introduction

Contamination is the presence of a (contaminant) in a material, in physical
minor and unwanted constituent body, in the natural environment, at a work

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place, etc. (Collins et al., 2001). Frequent environmental contaminants within microbiology laboratory create not only diagnostic dilemmas but also poses major risk for health care workers and patients (Konar, and Das, 2013; Rhame, 1998). Moreover, laboratory contamination is a marker of quality control of hospital disinfection and sterilization policy (Vesley, 2001).

The number of microorganisms transferred depends on the microbiological loading of the air and the exposure time. Many types of particle contamination can occur as like glass, rubber, plastic and un-dissolved particles (Douglas et al., 2001). Blood culture in Bacteriological laboratory is mostly victimised of laboratory contamination (Kirchhoff et al., 1985). Environmental contamination of clinical microbiology laboratories with vancomycin-resistant enterococci (VRE) has been previously described. Collins and colleagues demonstrated recovery of VRE on 10% of laboratory surfaces tested (Collins et al., 2001).

The use of aseptic techniques and other good microbiological practices achieves two very important objectives. These include the prevention of contamination of the laboratory by the organisms being handled, and the prevention of contamination of the work with organisms from the environment. These include the use of manipulation techniques that minimize the possibility of producing aerosols and to keeping the laboratory clean and tidy. Infection prevention protocols are effective in reducing the health care associated infections (Knox, 2010). The use of 70% propyl alcohol found to be effective in reducing contamination of Laboratory equipment's than other agents like detergents. (Alothman et al, 2009; Parmar, 2004; Nelson, 2006)

There are some contaminant levels which are applicable to facilities such as

diagnostic, research, clinical, teaching and production facilities that are working at a laboratory scale. Practical laboratory approaches to the workup of likely contaminants are therefore very important footstep to discriminate between the true pathogens and laboratory contaminants.

Current National Committee for Clinical Laboratory Standards guidelines (Wayne, 2002) recommend the use of *Enterococcus faecalis* ATCC 51299 for the quality control of culture media used in the detection of acquired aminoglycoside and glycopeptide resistance in enterococci. Commercial suppliers of commonly used automated susceptibility testing systems also recommend the use of this strain for quality control of their products (Robert, et al., 1992). The aim of this study is to identify the contamination level of different laboratory used equipment's and bacterial profile. Objective of our study was to isolate and identify the common laboratory contaminant bacteria to reduce false positive culture reports and to reduce laboratory acquired infections and also to maximize the true result comes from microbiology lab.

Materials and Methods

The study was conducted in the laboratory of Microbiology department of Kohat University of Science and Technology Kohat Pakistan from March 2014 to June 2014. The study was designed to investigate the common microbes present in lab environment causing different types of contamination.

Samples were collected from different areas of microbiology laboratory like air, cloth, floor, tables and incubator. The samples were inoculated on LB media and blood agar. The identical Bacterial colonies were sub cultured in to the respective growth media. The plates were incubated at 37°C to obtain pure cultures of

isolated bacteria. Then sub culture them and again incubated at 37°C. The liquid culture must be streaked out on an agar plate blood agar chocolate agar nutrient agar to determine if there are any contaminants (Robert et al 2009).

Gram staining was done to differentiate between Gram positive and Gram negative bacteria by following standard protocol of gram staining. Then to identify the presence of endospores in a bacterial sample, Spore staining was done. Special techniques for endospore staining include the Schaeffer–Fulton stain and the Moeller stain used to distinguish between the vegetative cells and the endospores by following standard protocol of gram staining (Nelson, 2006).

Results

Different bacterial strains were isolated from specified areas in the laboratory as shown in Table 1. Total four bacteria were isolated and identified from the laboratory area with various frequencies. The highest frequency 8 (36.36%) was *Staphylococcus epidermis*'s followed by *Bacillus subtilis* 7 (31.81%) and *Staphylococcus aureus* 4 (18.18%) while minimum frequency was recorded for *Depthriods* 3 (13.63%).

Biochemical Tests

Various biochemical tests were done on the basis of which different bacterial strains were identified as shown in Table 2. Total of four bacterial contaminants were detected that include *Bacillus subtilis*, *Staphylococcus Aureus*, *Staphylococcus epidermis*, *Depthriods*.

Discussion

Environmental contaminants of laboratories differ from laboratory to laboratory depend upon the work and experiment perform there and geographical

Then aerobic gram-positive cocci contaminants initially were identified based on colony characterization, hemolysis pattern and gram staining of the colonies. Further identification was made with Catalase test, Mannitol fermentation, and Coagulase test. For identification of Gram negative bacteria the following tests were done; catalase, oxidase, urease, indole, citrate utilization, lysine decarboxylation, glucose & lactose fermentation, gas & H₂S production and motility tests (Knox, 2010). All biochemical test reagents were purchased from Oxoid Ltd. Company, UK.

Statistical Analysis

Microsoft excel 2013 was used for the statistical analysis of the data. The results were obtained in rates (%).

Different studies were performed by researcher in many countries and institutions concluded different results. In this study we examined that in air samples, 3 (42.85 %) *Bacillus subtilis*, 1 (25%) *Stahylococuss aerous*, and 1 (33.33%) *Depthriods* while work done by (Konar and Das 2013) on air sample show result that only 6 *Bacillus subtilis* was present. The sample from cloths show that 2 (28.5%) *Bacillus subtilis*, 1 *Stahylococuss aerous*, 4 (50) *Staphylococcus epidermidis*, While work done by (Collins et al., 2001) on cloths show that only 1 *Staphylococcus epidermidis* was present. The sample from floor showed that 1 (12.5 %) *Staphylococcus epidermidis* and 1 (33.33 %) *Depthriods* while work done by Vesley et al. (2001). On Floor samples showed that only 2 *Bacillus subtilis* was present. The sample from table showed that 1 (14.25 %) *Bacillus subtilis*, and 2 (25 %) *Staphylococcus epidermidis*, while work done by (Alothman et al 2009).

Table 1. Frequency of various bacterial contaminants in laboratory area.

Samples	Number of isolates along with their percentage			
	<i>Bacillus subtilis</i> (No. of isolates)	<i>Staphylococcus Aureus</i> (No. of isolates)	<i>Staphylococcus epidermis's</i> (No. of isolates)	<i>Depthteriods</i> (No. of isolates)
Air	3 (42.85)	1 (25)	0	1 (33.33)
Clothing	2 (28.5)	1 (25)	4 (50)	0
Floor	0	0	1 (12.5)	1 (33.33)
Table	1(14.25)	0	2 (25)	0
Incubator	1 (14.25)	2 (50)	1 (12.5)	1 (33.33)
Total	7 (31.81)	4 (18.18)	8 (36.36)	3 (13.63)

Table 2. Identification of the isolated bacteria from laboratory area.

Gram stain reaction	Morphology	Oxidase	Catalase	Coagulase	Spore staining	Hemolysis pattern	Identified Bacteria
Positive	Rods	Positive	Positive	Neg	Positive	Alpha hemolysis	<i>Bacillus subtilis</i>
Positive	Cocci (Cluster)	Positive	Positive	Positive	Neg	Beta hemolysis	<i>Staphylococcus Aureus</i>
Positive	Cocci (Chain)	Neg	Positive	Neg	Neg	Non hemolytic	<i>Staphylococcus epidermis's</i>
Positive	Polymorphic	Neg	Neg	Neg	Neg	Non hemolytic	<i>Depthteriods</i>

On Table's sample showed that 1 is *Staphylococcus Aureus*, 2 *Staphylococcus epidermidis* and 4 *Depthteriods*. The sample from incubator showed that 1 (14.25 %) was *Bacillus subtilis*, 2 (50 %) *Staphylococcus aerous*, 1 (12.5 %) *Staphylococcus*

The biochemical test showed that all bacteria are gram positive while (Kimberley Christopher and Elsa Bruno) also use gram staining technique for identification of bacteria. *Staphylococcus aerous* is morphologically cocci (round shape) and biochemically it is Oxidase, Catalase and Coagulase positive, while (Identification of *Staphylococcus* species, *Micrococcus* species and *Rothia* species) also show same result about *Staphylococcus aerous*.

epidermidis, and 1 (33.33 %) *Depthteriods*. So in our work total *Bacillus subtilis* 7 (31.81 %), *Staphylococcus Aureus* was 4 (18.18 %), *Staphylococcus epidermidis* was 8 (36.36 %) and *Depthteriodsis* 3 (13.63 %).

Staphylococcus epidermidis morphologically cocci (round shape) and biochemically it is Oxidase, Catalase, Coagulase, and spore staining is negative, while (Identification of *Staphylococcus* species, *Micrococcus* species and *Rothia* species) also show the same result about *Staphylococcus epidermidis*, our result showed the similarity with the result of Nelson et al (2006) and Robert et al. (2009).

Conclusion

Staphylococcus epidermis and aerobic spore bearers, i.e. *Bacillus subtilis* was the common contaminants in Microbiology laboratory. Rate of contamination recorded in this study was higher than any set

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