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Ubiquitous adulterants in the microbiological investigations of tertiary care centre in mid-Nepal

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ABSTRACT

Background: The bacterial agents can be isolated even from the aerosol, hands, cloths, and floors of the laboratory. So, the microbiological agents isolated from these areas can act as contaminant in the microbiology laboratory and may be an obstacle in the routine processing and culture of the specimen. This leads to the false positive results and false negative results. As a consequence of which, inaccurate therapeutic regimens are prescribed to the patients, this causes unusual serious effects in the other unaffected parts of the body. **Aim:** The purpose of this study was to illustrate the bacterial agents that are responsible to act as a contaminant in the laboratory. **Methods:** The samples were collected from the floors, working surface and aerosol of the laboratory whereas the samples were also obtained from the hands and cloths of the laboratory staffs of Chitwan Medical College and Teaching Hospital. The isolates were identified by the standard microbiological protocols applied routinely in the lab. **Results:** Out of 180 samples, growth was observed in 38 of them. Among 38 positive samples, *Micrococcus* spp. was isolated in 14 (36.8%) followed by *Bacillus subtilis* in 7 (18.4%). *Diphtheroids* were observed in 4 samples (10.5%). *Staphylococcus epidermidis* was found in 6 (15.8%) samples whereas *Staphylococcus aureus* were isolated in 5 (13.1%) samples. **Conclusion:** The bacteriological agents can be isolated from various areas of laboratory and may act as a contaminant which might degrade the quality or the accuracy of the reporting of the results.

Introduction

The novel techniques and standard protocols applied in the microbiology laboratory provides the accurate diagnosis of the patients. However there is a high potentiality of the occurrence of false positive and false negative results. These errors might happen during cross-contamination, use of non-sterile reagents and equipments, mishandling of specimen, storage, packaging and delayed transportation of the sample to the laboratory. Sometimes false positive and false negative results could not be identified [1].

Microbial contamination becomes an obstacle for laboratory workers working with

microbial cultures. The ubiquitous microbial adulterants in the microbiology laboratory include bacteria, fungi, yeasts and molds. They degrade the quality of result and even lead to the loss of some valuable strains cultured in the lab. Moreover, it could pose a threatening risk to the health of the patients, health workers and to the people of a single community too. False positive and false negative results leads to the misdiagnosis of the patients and inaccurate prescription of the therapeutic regimens. Moreover, it could cause serious complications in the other unaffected parts of the body [2-4].

The purpose of this paper was to isolate and identify the common adulterants in the laboratory in

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order to maximize the true positive results and minimize the false positive results.

Materials and Methods

The research was conducted in bacteriology laboratory of Chitwan Medical College Teaching Hospital, Bharatpur, Chitwan, Nepal over a period of three months from January 2020 to March 2020.

Specimen collection

A total number of 180 samples were collected by using swabs from various areas of laboratory like atmosphere, floor surfaces, working bench, hands and clothing of the laboratory workers. For the isolation of atmospheric bacteria, the nutrient agar plates, blood agar plates and Mac-Conkey agar plates were kept open in upright position so that the bacteria could adhere to the media.

Processing of specimens

The collected swab specimens were processed according to the method described in study carried out by Konar et al. [5]. First the collected swab specimens were cultured on solid culture media such as Nutrient agar, MacConkey agar, Cysteine Lactose Electrolyte Deficient (CLED) agar, as well as on Blood agar, then cultured in liquid medium (Brain Heart Infusion broth). The inoculated plates were incubated at 37 °C for 24 hours. The liquid medium was incubated for up to 4 days for any possibility of bacterial growth and in case of any positive bacterial growth (turbidity), sub-cultures on solid culture

media were performed. The colony morphology and characteristics were studied from the bacterial growths if any. The bacterial colonies were also subjected for Gram-staining and biochemical tests such as catalase, oxidase, citrate, urease, SIM (sulfide, indole, motility), and TSI (Triple Sugar Iron) tests as recommended by American Society for Microbiology [6].

Ethical approval

This study was approved by the Institutional Review Committee (IRC) of Chitwan Medical College and Teaching Hospital, Bharatpur, Chitwan, Nepal.

Results

As shown in **table (1)** and **(2)**, out of 180 samples, growth was observed in 38 of them. Among 38 positive samples, *Micrococcus* spp. was the most commonly isolated contaminant (14) followed by *Bacillus subtilis* (7), *Staphylococcus epidermidis* (6), *Staphylococcus aureus* (5), *Diphtheroides* (4), and *E. coli* (2).

The majority of the contaminants were isolated from the hands (15), followed by air (9), floor surface (7), and clothing (4) as shown in **table (1)**. Among the bacterial isolates, most of the *S. aureus* isolates were recovered from hands whereas majority of the micrococcus isolates were recovered from air and floor surfaces (**Table 1**).

Table 1. Number of bacterial isolates from various samples.

Samples	No. of samples	<i>Staphylococcus aureus</i>	<i>Staphylococcus epidermidis</i>	Diphtheroids	<i>Bacillus subtilis</i>	<i>Micrococcus</i>	<i>Escherichia coli</i>	Total
Air	36	0	0	0	4	5	0	9
Floor surface	36	0	0	0	2	5	0	7
Working surface	36	0	0	0	1	2	0	3
Hands	36	5	4	3	0	1	2	15
Clothings	36	0	2	1	0	1	0	4
Total	180	5	6	4	7	14	2	38

Table 2. Percentage of bacterial isolates from various samples.

Name of isolates	Number	Percentage
<i>Staphylococcus aureus</i>	5	13.1
<i>Staphylococcus epidermidis</i>	6	15.8
Diphtheroids	4	10.5
<i>Bacillus subtilis</i>	7	18.4
<i>Micrococcus</i>	14	36.8
<i>Escherichia coli</i>	2	5.3
Total	38	100

Discussion

In the present study, the potential bacterial contaminants found in the hospital settings were investigated by collecting specimens from the floor surface, working surface, clothing and hands.

The hands are considered as the primary vector for the transfer of microorganisms from one individual to other and the indoor environment contamination with these microorganisms may lead to the spread of nosocomial infections among susceptible patients [7]. Moreover, the pathogens found in air environment originating from the patients itself through the droplets produced during coughing [7] can colonize the patients care settings [8,9].

The microbial contaminants differ from one geographical region to the other and from one laboratory to the other. These solely depends upon the techniques and good practices applied in the lab and even on the infection control policy of the health centers. The study carried out by **Konar et al.** finds out the *Bacillus subtilis*(75%) to be a major contaminant followed by *Micrococcus spp.*(66.67%) which is different to our study as this study finds out the *Micrococcus* to be major contaminant (36.8%) followed by *Staphylococcus epidermidis*(15.8%) [5]. The study carried out by **Veena et al.** illustrates that the Coagulase negative *Staphylococci* was the major contaminant followed by *Corynebacterium* species.(38%) as a second major contaminant, which is also not in the correlation to our study [10]. The study conducted by **Muhammad et al.** finds out the *Staphylococcus epidermidis* (36.36%) to be the most frequent contaminant 8 (36.36%) followed by *Bacillus subtilis* (31.81%) and *Staphylococcus aureus* 4

(18.18%) while Diphtheroids (13.63%) to be the less frequent bacteria which differs from our study [11].

Conclusion

This study finds out that the microbial contaminants could be isolated from the various areas of laboratory like cloth, floors, hands, working surfaces, air, etc. and can be the source of contamination in our cultured plates. So, besides these, it also suggests that the microbial contamination should be supervised before processing out the specimens in the laboratory. Appropriate infection control guidelines should be implemented in all the health centres in order to avoid the misdiagnosis of the patients and inaccurate prescription of the therapeutic drugs. The proper laboratory practices may be followed to reduce the microbial contamination and their serious hazards to the health workers, patients and even to a whole community. The microbial contamination obviously interferes results reported in the labs. So, the working areas including the atmosphere, floors and walls should also be disinfected before processing the specimens.

Conflict of interest

No conflict of interest among the authors and coauthors.

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