



Original Research Article

Study of bacteria on computer's mice and keyboards

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ABSTRACT

The present study was conducted to isolate and identify pathogenic microorganisms on the external surface of computer keyboards and computer mice. For this purpose, total 300 samples were collected from different computer labs of LCWU, Lahore, Pakistan. The samples collected were cultured with Nutrient Broth, Nutrient Agar and for further identification they were cultured on the selective media. All 300 samples were found contaminated with pathogenic bacteria (*E.coli*, *Salmonella*, *Shigella*, and *Staphylococcus*). *E.coli* dominated the isolates. The second most common bacterial growth in all samples was Gram-positive *Staphylococcus*. Potential pathogens isolated from all specimens were: *Staphylococcus aureus*, *Salmonella*, *Shigella* and *Pseudomonas* spp. and Gram negative bacilli. Results indicate that computer 'mice and keyboards showed 100% contamination in comparison with other objects. The presence of pathogenic and commensal bacteria on these objects indicates that they might act as environmental vehicles for the transmission of potentially pathogenic bacteria.

Keywords

Computer's keyboards (CK) and computer's mice (CM)

Introduction

Contamination occurs everywhere including environment and all its objects. Computer's keyboards and mice are the most open surface parts of computer which show 100% contamination. This study has demonstrated that microbial contamination of multiple-user computer keyboards may be a common mechanism of transfer of potentially pathogenic bacteria among users. Computers continue to have an increased presence in almost every aspect of our occupational, recreational and residential environments. In various

university environments, students have indicated 100% access to computers, 92.1% regularly use internet and 73.3% regularly use e-mail (Palmer and Bray, 2001). As the population of such facility increases, there is need to recognize that computer equipment may act as a reservoir for the transmission of potential hazardous or pathogenic microorganisms (Hartman *et al.*, 2004). The ability of a computer to act as fomites has been previously documented in healthcare (Huber and Pelon, 2005) and hospital environment (Bures *et al.*, 2000).

In work place, contamination of the office environment (including the computer keyboard and mouse) with bacteria is also recognized (Hirsch, 2005). The increased availability of multiple-user computers in the university setting means that these items or equipment are handled by numerous users on a daily basis. Given that computers are not routinely disinfected, the opportunity for the transmission of contaminating microorganisms is potentially great. Our understanding of the ubiquity of microorganism in the environment is developing, but the risk or hazard of contamination posed by the computer keyboards and mice is not yet fully understood. Most people do not realize that microbes are found on many common objects outdoors, in their offices, and even in their homes. Such objects include; playground equipments, ATM keyboards, kitchen sinks, office desks, computer keyboards, escalator handrails, elevator buttons and with the spread of supermarkets and hypermarkets the shopping carts handles. All of the latter objects are places that are most touched by the bare hands of people who are in various hygienic conditions. People believe that microbes are only present in research labs or in hospitals and clinics and thus they have a misleading feeling of security in other places. Lack of knowledge about where germs prowl could be the cause of health problems. In fact 80% of infections are spread through hand contact with hands or other objects (Reynoldset *al.*, 2005).

Reynolds used an invisible fluorescent tracer for artificial contamination of public surfaces, they found that contamination from outside surfaces was transferred to 86% of exposed individual's hands and 82% tracked the tracer to their home or

personal belongings hours later (Reynolds *et al.*, 2005). Enterococci have been found to survive in dry conditions and on various fabrics utilized in the healthcare environment. Infectious doses of pathogens may be transferred to the mouth after handling an everyday contaminated household object (Rusin *et al.*, 2002).

Recently (Ulger *et al.*, 2009) have demonstrated that health care workers' hands and mobile phones were contaminated with various types of microorganisms and concluded that mobile phones used in daily practice maybe a source of nosocomial infections in hospitals. Scientific information about the occurrence of bacteria on various objects outside the health care facilities is very little and needs to be enriched in order to educate people on the necessity of improving the habit of hand washing to reduce microbial transmission. The aim of this study was is to investigate the presence of bacteria on 4 different objects (computer keyboards and computer mice, elevator buttons and shopping carts handles) that are frequently used by people in the city of Jeddah, Saudi Arabia. (Ulger *et al.*, 2009)

In a discussion of the bacterial niche of a keyboard, so much depends on the user and what bacteria and contaminants a user brings to the device. This section will focus on niche conditions that present itself as optimal for bacteria in an environment exposed to active practice of medicine. Significant amount of bacteria on keyboards in healthcare environments is transferred through wet gloves, contaminated gloves, or poor hygiene from healthcare specialists. Bacterial transmission results from tapping on the keys and regular usage of the device, which may incur contaminants such as

blood, secretions, or other various sticky substances in such a healthcare environment (Fukata *et al.*, 2008). From tests carried out, 95% of cultures from keyboards tested positive for microorganism though most were simple skin flora (Schultz, Maureen *et al.*, 2003). The focus of research has been on pathogenic bacteria that pose threats to nosocomial infections.

Bacteria that are often found in a healthcare environment include coagulase-negative *Staphylococcus*, *Bacillus* species, *Corynebacterium* species, streptococci, *Clostridium perfringens*, *Enterococcus* species, *Staphylococcus aureus*, gram negative bacteria, and fungi (Rutala, William *et al.*, 2006). Of significant importance in healthcare environments involve antibiotic resistant strains of microbes which include *Staphylococcus aureus*, Vancomycin-resistant enterococci, and methicillin-resistant *Staphylococcus aureus* (MRSA). The capability of these bacteria to survive for more than 24 hours further increases their chances of contamination in other places.

The environmental conditions vary depending on temperatures around the keyboard and whether or not the keyboard is on a laptop. If the keyboard is on a laptop it could possibly provide heat and moisture for long enough durations to have an effect on bacteria such as *Enterococcus*, which is known to survive a wide range of environmental conditions. Studies have shown, however, that the presence of serum or albumin (known contaminants on keyboards touched by wet gloves), and a low temperature, with high humidity results in longer lifetime of bacteria on contaminated surface. Many nosocomial pathogens can also survive on

dry inanimate surfaces for months (Kramer *et al.*, 2006).

Coagulase-Negative *Staphylococcus* e.g. *Staphylococcus aureus* usually found on skin or in the nasal environment and only survives on dry skin on the outside of the body. Appears on keyboards quite a bit as a result of usage. Methicillin resistant strain of *Staphylococcus aureus* found on keyboards a high percentage of the time in hospital environments which can cause infections in patients (Fukata *et al.*, 2008). *Clostridium perfringens* usually found in human gastrointestinal tracts and environments such as sewage and soil, however, in a healthcare environment can cause gas gangrene. Probably will not survive long on a keyboard as its primary target is living tissue. Found on keyboards at lesser degrees.

Enterococcus bacteria are usually found in the bowel and are known to be able to survive adverse conditions that other bacterial usually won't grow in. They are known to survive at temperatures of 60C and in anaerobic conditions with varying degrees of acidity. *Enterococcus* species represents some of the highest rates of appearances in hospital environment keyboards (Hartman, *et al.*, 2004). *Corynebacterium* commonly found on human skin and mucous membranes. *Streptococcus* mostly anaerobic while some are facultative anaerobes, they do not carry out oxidative phosphorylation and can survive in more acidic environments which might be present on keyboards. Medically significant streptococci require lots of amino acids, vitamins, and nutrients thus are not normally found isolated in an environment.

In this study Gram +ve bacteria were more frequently isolated from all surfaces compared to Gram -ve. This could be in part due to the fact that survival of Gram +ve species on laminate surfaces is greater than that of Gram negative organisms (Scott and Bloomfield, 2008). However, Gram +ve and Gram -ve bacteria have been shown to have similar transfer rates from laminate surfaces to finger tips (Scott and Bloomfield, 2008). Normal skin is inhabited with two categories of bacteria: transient and resident. Resident flora, which are attached to deeper layers of the skin, are more resistant to removal by routine washing. Coagulase-negative staphylococci and Gram +ve diphtheroids are members of this group (Boyce and Pittet, 2002). On the other hand, transient flora colonizes the superficial layers of the skin, and is more amenable to removal by routine hand washing (Boyce and Pittet, 2002). Domestic and public computer keyboards and mice were swabbed and cultured. The swabbed areas were the keys mostly pressed like the space bar, the Enter and Backspace buttons. 100% of Internet café's computers were found to be contaminated.

The present study showed that microbial contamination occur on computer surfaces located in a university setting and may reflect the multiple-user environment where the possibility of contamination by individuals who are carriers of bacteria such *Staphylococcus aureus* is greater and the isolation of viable microorganisms suggest that the species present are able to persist for a period of time on these surfaces. It is suggested that computer keyboards and mice in institutions may act as a vehicle for the transmission of pathogenic organisms (Anastasiades, et al., 2009).

The computer's keyboard and a mouse have a very dynamic environment. Food and drinks are commonly consumed in front and on top of the keyboard. This provides a constant source of food for anything that may be living there. Since the keyboard is constantly in contact with human hands, there will constantly be a stream of things leaving the hands and entering the keyboard or of course, leaving the keyboard and entering the hands. In the former case, bacteria from different niches could be transferred over if the hands have not been sanitized. In the later case, already preexisting bacteria will start to grow will transfer onto the hands and then onto the new surfaces that the hand will come into contact with. There are many communities that come into contact with the keyboard. In general, the bacteria that lives on our skin, fingernails, hands, and anywhere the hands have been are likely to transfer new bacteria over to the keyboard. In a place where there is a lot of people moving in and out, such as a hospital or office, there is likely to be a good number of people that are sick, and through them comes the new bacteria that will eventually settle on the keyboard through the air or from physical contact.

Cleaning our keyboard and mice regularly is another smart solution that most people ignore; only about half of computer users in the average office environment clean their computer keyboards at least once a month. Of course, sharing a keyboard with multiple people makes it a much more dangerous surface when it comes to passing diseases. If we use our own keyboard and mouse and nobody else uses it, then the chances of that keyboard and mouse serving as a method of transmission is fairly small, but where sharing keyboards is concerned there is a higher probability of transmission occurring. We

can clean in between the keys of our keyboard with compressed air (i.e. canned air) on a monthly basis, and we can also use canned air to blow out the fans and vents on our computer regularly too. We can also wipe the surface of the keys and keyboard with anti-bacterial wipes regularly for the best protection

Materials and Methods

The research was focused on the microbial studies of pathogenic bacteria on computer's mice and keyboards collected from different computer labs of LCWU, Lahore, Pakistan. Total 300 samples were collected from different computer labs of LCWU to test the presence of pathogenic bacteria. First group of 150 computer keyboards were collected from December 2012 to February 2013. And second group of 150 computer mice were collected from April 2013 to June 2013. The samples were collected with the help of sterile cotton swabs and were placed in a sterile plastic box.

Isolation of various bacterial contaminants from these two objects (CM and CK) was performed through standard techniques. Briefly, sterile water moistened swabs were wiped firmly over the entire surface of the specific object. Each swab was placed in 2 ml of Nutrient broth in a sterile test tube, and vortexed for one minute. In the same manner, all the 300 samples of dust containing sterile cotton swabs were dipped separately into the nutrient broth and were left in the shaker for overnight. After 18-24 hours of inoculation, the nutrient broth medium become turbid, turbidity ensured the presence of certain bacteria in the samples. For morphological identification of bacterial colonies, nutrient agar plates were prepared. Small amount of culture was taken with the help

of inoculating loop from the nutrient broth medium and were streaked on the agar plates. Different types of bacterial colonies appeared on the Nutrient agar plates. The samples collected were cultured with Nutrient Broth, Nutrient Agar and for further identification they were cultured on the selective media. Pure colonies of isolates were identified and characterized using standard microbiological technique. All 300 samples were found contaminated with pathogenic bacteria (*E.coli*, *Salmonella*, *Shigella*, and *Staphylococcus*).

Results and Discussion

The main objective of the present microbial study was to isolate and identify the pathogenic microorganisms on the external surface of computer's mice and keyboards to create public awareness about the health hazards resulting from these pathogenic microorganisms. To conduct this study, total 300 samples were collected from different computer labs of LCWU, located in Lahore, Pakistan. All the samples were first cultured on nutrient broth and nutrient agar, which ensured the presence of certain pathogenic microorganisms. For further confirmation and identification, the culture from the nutrient agar was streaked on different selective media and the presence of pathogenic bacteria like *E.coli*, *Salmonella*, *Shigella*, *Staphylococcus*, and *Streptococcus* was confirmed. *E.coli* dominated the isolates. The second most common bacterial growth in all samples was Gram-positive *Staphylococcus*. Potential pathogens isolated from all specimens were: *Staphylococcus aureus*, *Salmonella*, *Shigella*, *Pseudomonas* spp. and Gram negative bacilli.

To isolate these pathogenic

microorganisms, cotton swabs containing computer's mice and keyboards dust were dipped in the nutrient broth medium and the turbidity produced in the medium, (after 24 hours incubation with shaking) indicated the presence of certain microorganisms. To confirm their presence the culture was taken from the nutrient broth and streaked on the nutrient agar plates as shown in fig 1. Different bacterial colonies were obtained which were further confirmed by culturing them on the selective media

The selective media which were used for identification of pathogenic microorganisms include Sorbitol MacConkey Agar, Mannitol Salt Agar, Eosine Methylene Blue Agar, Hektoen Enteric Agar and EC media. All the selective media showed the growth of respective bacteria.

Growth on Eosine Methylene Blue agar (EMB)

This media is used to differentiate between the colonies of lactose fermenting and non-fermenting microbes. It is selective for gram negative bacteria like *E.coli* and inhibits the growth of *Salmonella*, *Shigella* and *Staphylococcus*. EMB agar was prepared and streaked with culture containing bacteria, after 18 hours of incubation it was observed that lactose fermenting bacteria appeared like a nucleated colonies with dark centers.

On EMB *E.coli* gives a distinctive metallic green sheen color (due to the metachromatic properties of the dyes, *E. coli* movement using flagella, and strong acid end-products of fermentation. This medium has been specifically designed to inhibit the growth of gram positive bacteria. EMB agar is useful in isolation and differentiation of the various gram-

negative bacilli and enteric bacilli, generally known as coliforms and fecal coliforms respectively. The bacteria which ferment lactose in the medium form colored colonies, while those that do not ferment lactose appear as colorless colonies as shown in fig 2.

Growth on sorbitol macconkey agar (SMAC)

This media is used for the identification of *E.coli* in the respective culture. *E.coli* is a pathogenic bacterium which causes not only diarrhea but also Hemolytic Uremic Syndrome (HUS) in acute condition. Some other infections like neonatal meningitis, urinary tract infection (UTI), gastroenteritis and respiratory illness are also in common observation. Sorbitol MacConkey agar is a medium which contains sorbitol instead of lactose and *E.coli* has an ability to ferment lactose but does not ferment sorbitol. SMAC medium was prepared and streaked with the bacterial culture, after 18 hours of incubation light pink color colonies were observed which confirmed the presence of *E.coli* in the culture.

Growth of gram-positive bacteria (e.g. *Staphylococcus aureus*) is inhibited by the crystal violet dye and bile salts in the media. While gram-negative bacteria like *Salmonella* grow on MacConkey agar, but do not ferment lactose (media appears yellow to light pink in color & colonies are colorless) as shown in the fig 3.

Growth on hektoen enteric agar (HEA)

This media is used for identification of *Salmonella* and *Shigella spp.* Both these bacteria are pathogenic and cause variety of diseases in humans. *Salmonella* are bacteria that cause diseases (gastroenteritis, typhoid fever, paratyphoid

fever) in humans. They are transferred to humans by many routes (for example, unwashed fruits, vegetables and nuts, uncooked or undercooked meats and eggs, contaminated water). *Shigella* is highly contagious and it causes bacillary dysentery and diarrhea.

The growth of *E.coli* is inhibited on this medium while *Shigella* showed a very good growth with green colonies and *Salmonella* appeared as green colonies with black centers. Results of Hektoen enteric agar as shown in fig.4. Cultural response on Hektoen Enteric Agar at $35\pm 0.2^{\circ}\text{C}$ after 18-24 hours incubation is as follows:

Growth on mannitol salt agar (MSA)

MSA is a differential and selective media. It is selective because its high salt concentration (7.5 %) inhibits the growth of most bacteria. However, *Staphylococcus* is able to tolerate this high salinity. MSA is differential because it contains the sugar mannitol and phenol red, a pH indicator. When mannitol is fermented, acid products are produced and the pH drops. Phenol red is yellow in color below pH 6.8. Thus, mannitol fermenters such as *Staphylococcus aureus* will have a yellow halo around them. Mannitol non-fermenters such as *Staphylococcus epidermidis* will leave the MSA media unaltered (pink). When mannitol is fermented by a bacterium, acid is produced, which lowers the pH and results in the formation of a yellow area surrounding an isolated colony on MSA. A non fermenting bacterium that withstands the high salt concentration would display a red to pink area due to peptone breakdown. Coagulase-positive *Staphylococci* produce yellow colonies with yellow zones, whereas coagulase-

negative *Staphylococci* produce small pink or red colonies with no color change to the medium as shown in the fig.5.

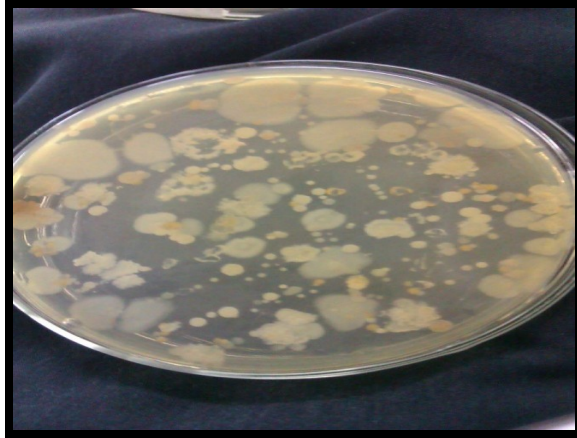
Growth on selective media (EC media modified with novobiocin)

This media is used for identification of *E.coli*. The media was prepared and streaked with the bacterial culture, after the incubation of 24 hours it was observed that yellow gold color of media turned pink. This pink color ensured the presence of *E.coli*. EC Medium, Modified with Novobiocin is used for the selective enrichment of *Escherichia coli* O157:H7. This Medium is made in an effort to improve the methods for the detection of the coliform group and *E. coli*. This medium consists of a buffered lactose broth with the addition of 0.15% Bile Salts Mixture. Growth of spore-forming bacteria and fecal streptococci were inhibited by the bile salts. EC Medium, Modified with the addition of Novobiocin was first described by Okrend and Rose (1990). Growth in EC medium modified with novobiocin is demonstrated as an increase in turbidity as shown in fig.6.

Gram staining of pathogenic bacteria

The gram stain is used to categorize bacteria on the basis of their forms, sizes, cellular morphologies, and gram reactions. However, in a clinical microbiology laboratory, it is a critical test for the rapid diagnosis of infectious agents and help in assessing the quality of clinical specimens. It was originally developed by Danish bacteriologist Hans Christian's gram in 1884. Gram stain makes separation of all bacteria into two large groups; those which retain the primary dye (gram-positive) and those that take the color of counterstain (gram-negative). The primary dye is crystal violet and the secondary dye is Safranin.

Fig.1 Growth of pathogenic bacteria



On Nutrient agar

Fig.2 EMB media showing colonies of lactose fermenting pathogenic bacteria (*E.coli* with dark centers)

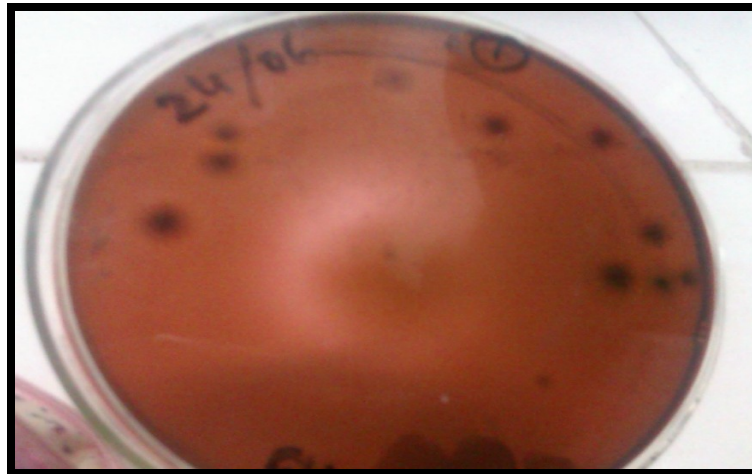


Fig.3 MacConkey Agar plates showing growth of pathogenic *E.coli* O157:H7

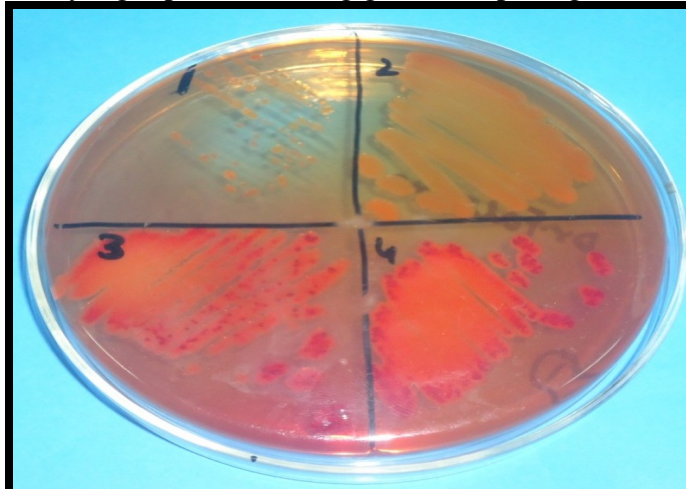


Fig.4 Hektoen Enteric Agar plates showing growth of pathogenic *Salmonella* and *Shigella*

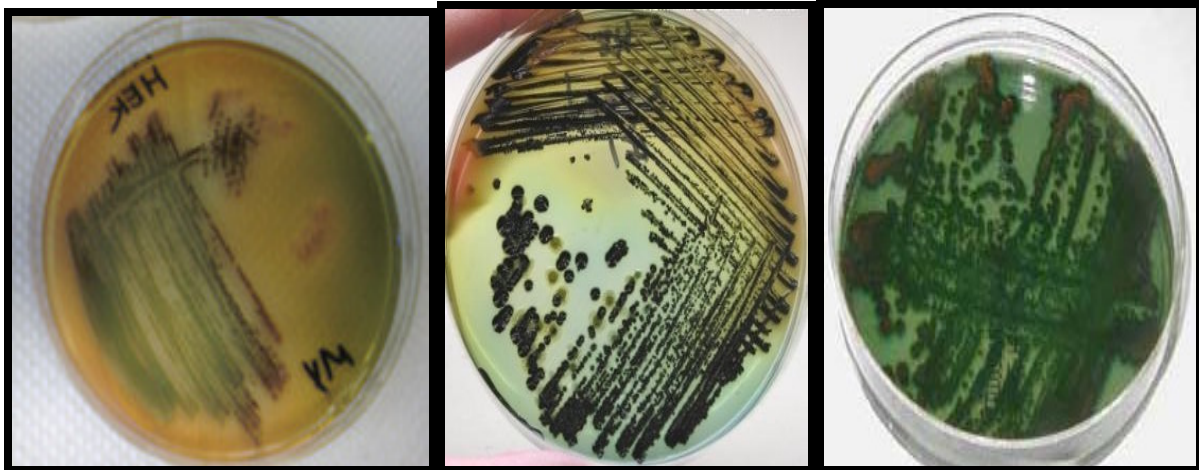
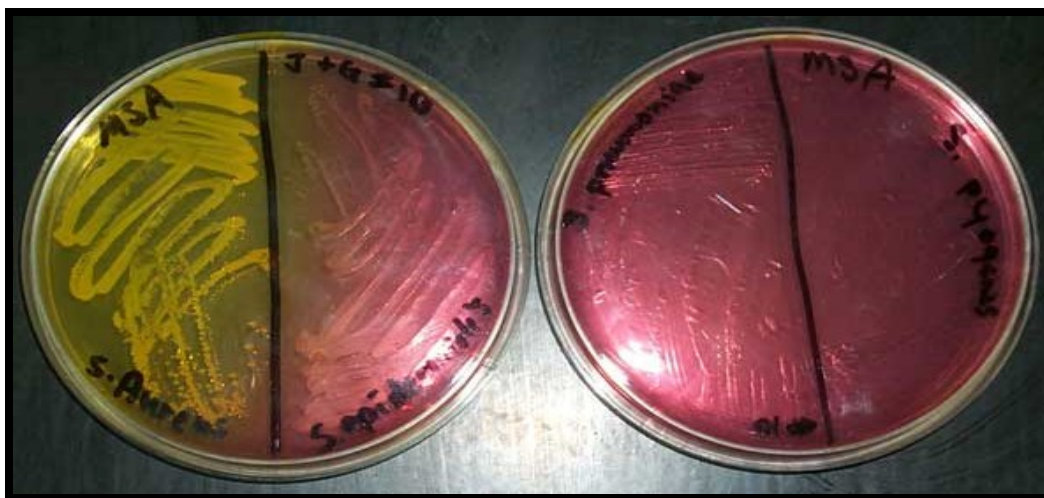


Fig.5 Mannitol Salt Agar plates showing growth of pathogenic *Staphylococcus aureus*



Gram staining results

Analysis of gram-stained smears involves consideration of staining characteristics and cell size, shape and arrangement. These properties can be influenced by culture age, media, incubation, atmosphere, staining methods and the presence of inhibitory substances. Results are shown in the figure below. The presence of pathogenic bacteria on computer's mice and keyboards indicates

not only the poor environmental conditions but also the poor environment hygiene. The present study provides clear evidence that *E.coli*, *Salmonella sp.*, *Shigella sp.* and *Staphylococcus sp.* are frequently occurring bacteria in our environment, which not only make our environment unhealthy but also spread number of diseases. The presence of these bacteria on the keyboards and mice makes their external surface entirely unhygienic as a result when they are used by humans

make them ill or may cause death. Computer's mice and keyboards are the very important source of spreading these

Gram staining had also confirmed the presence of different pathogenic microorganisms on the external surface of computer's keyboards and mice. Under microscope different colonies were observed and were differentiated on the basis of their size, shape and color. The fact mentioned above clearly indicates that computer's mice and keyboards are the vital source of spreading different pathogenic microorganisms to humans and causes number of serious diseases. The main objective of the present study was to raise the awareness among the people about the deleterious effects of pathogenic microorganisms and computer's keyboard and mice. The presence of these deleterious bacteria makes our environment unhealthy.

Environment of Pakistan is favorable for the growth of pathogenic microorganisms (*E.coli*). It provides all the suitable conditions for them to nourish and prosper. To make our environment free of these bacteria, certain preventive measures should be taken.

In the present study, all the collected samples of dust containing cotton swabs from computer's mice and keyboards were found to be contaminated with human pathogenic bacteria, samples of dust were collected from different computer's labs of LCWU Lahore, Pakistan. Very effective procedures were used for the isolation and identification of bacterial colonies. Different selective and differential media were used for the isolation and identification of bacterial colonies. It was concluded that external surface of computer's keyboard and mice contain

pathogenic bacteria to humans and spreads skin borne illness.

pathogenic bacteria which are lethal to human life and they may cause harm or immediate death. So, there is a need for the eradication of dust from our daily used objects such as keyboards and mice to avoid the spread of pathogenic bacteria that causes very serious diseases in humans. This study will be helpful for the establishment of detection methods for pathogenic bacteria.

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