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Identify the Different Micro-Organisms and to Study their Effect

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Abstract: *The present study was conducted to isolate and identify pathogenic microorganisms on the external surface of Keyboard, ATM Machine. For this purpose, we took sample of computer keyboard and 5 sample of ATM machines and Samples were collected from computer keyboard and atm machine with normal saline rinsed swab. Samples were immediately cultivated on Nutrient agar plate and MacConkey agar and then we have to see the growing bacteria and identified based on their morphology and biochemical properties. Approx. maximum number of computer Keyboard and Atm machines were contaminated with bacteria and fungi. The must contaminated Pertained to gram positive bacteria and in the computer, keyboard were must isolated bacteria were Coagulase negative staphylococcus. The highest contamination rate was found on computer coagulase negative Staphylococcus. The highest contamination rate was found on computer keyboard. This study demonstrates that monitoring inanimate surface and considering these surfaces as source of nosocomial infection is necessary.[1] The results showed the presence of increased bacterial count subsequently, most pathogens on characterization revealed the genus of the particular organisms E. coli, Pseudomonas, Staphylococcus aureus, Klebsiella, Micrococcus, Salmonella and Serratia. The study showed the potential hazard inherent in ATM machine usage and draws attention to our level of hand hygiene compliance.*

Keywords: Contamination on computer keyboard, ATM machines pathogenic bacteria

I. INTRODUCTION

Microorganism occurs nearly everywhere in nature. They present in the air currents on earth surface, Mountaintops, bottom of the ocean, soil, water bodies, etc microorganisms occur most abundantly where they found moisture and a temperature suitable for their growth and multiplication. [3] Since the condition that favor the survival and growth of many microorganisms are those under which people normally live it is inevitable that we live among a multiple of microbe. Microorganisms are ubiquitous and have an amazing ability to adapt to new environments and further multiply in large numbers within a limited time with this interesting fact in mind, the ready familiarity of microbes with hardware interfaces such as cyber appliances and its user's calls for carrying out experimental studies to show the linkages between the three. The United State Centre for disease control (USCDC) in 2005 found out, that microbes could find exchange between contaminated hands and cyber appliances such as the surface of automated teller machines.[4] The ATM in question is a telecommunication device that aids the customers of banks to carry out transactions with ease, irrespective of time or place. The use of hardware interfaces such as the

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keyboard, mouse, printers and ATM keypad has greatly expanded over the past few years with the development of various forms of computer-based management applications. With these wild growth has consequently led to regular and unrestricted sharing of interfaces among users. With the ease at which microorganisms acquired from the human microflora or as transient organisms from the environment, and previous accounts of cross contamination of microorganisms, it is readily seen that pathogens could be transferred among users who share interfaces. Many Gram-positive bacteria such as *Enterococcus spp*., *Staphylococcus aureus* and *Streptococcus pyogenes* and Gram-negative bacteria such as *Acinetobacter spp*., *Escherichia coli, Klebsiella spp., Pseudomonas aeruginosa and Shigella spp*. Can survive for months on surfaces It has been observed that microbial contaminations are limitless especially in developing countries where most users of automated teller machines (ATM), Computers are are largely ignorant of the potential hazards they face each time when they use such kind of devices.[5]

II. MATERIALS AND METHODS

Study Area: This study was carried out in ZSCT's Thakur shyamnarayan degree college, Kandivali East, Mumbai. Different labs and places was used for the sample collection. The reason for this was that it had many labs and system and the ATM machines sample was taken from the Borivali station ATM machines dispensing notes.

Location of ATM

1. IDBI Bank Atm (Borivali station)
2. Andhra Bank Atm (Dattapada Road Borivali West)
3. Canara Bank Atm (Siddhart Nagar Borivali West)
4. Kotak Mahendra Bank (90 FT Road Thakur Complex Kandivali East)
5. Axis Bank (90 FT Road Thakur Complex Kandivali East)

Different times were used for the collection; early hours of the morning and peak afternoon periods. This bank was selected based on the fact that it had 2 ATM machines, dispensed currencies and was the most visited ATM machine on Campus at the time of this study.

Sample Collection and Processing: Swabs from the investigated surface of 5 computer keyboard and 5 samples from the ATM at different times were collected using disposable sterile cotton swab. Keyboard samples swab were taken in the Microbiology laboratories and E-library of Thakur shyamnarayan degree college and 5 Atm samples were taken nearby Borivali station. Swabs were collected by through rotating a cotton swab on the surface of the back of the keypad, touch screen and both the side of the and swab for the keyboard Were carried out in the same for the ATM also.

Bacterial Inoculation: The inoculation of which was involved was the direct streaking of the cotton swab sticks on nutrient agar and on MacConkey agar in petri dishes each labelled according to date and source of sample code. The streaked sample was then incubated for 24 hours at 37°C after which the colonies were observed.

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Materials: The materials used include glass wares such as MacCartney bottles,Nutrient agar bottle, beaker, conical flasks, measuring cylinder, glass slides, inoculating wire loop, aluminium foil, cotton wool, swab sticks and spirit lamp, Autoclave, Incubator etc.

Media/Agar:

- MacConkey agar
- Nutrient agar
- Composition of MacConkey agar: (peptone 17g, proteose peptone 3g, lactose 10g, bile sales 1.5g,Sodium chloride 5g, agar 13.5g, neutral red 0.03g, crystal violet 0.001g, distilled water 1L, final Ph 7.1)
- Composition of Nutrient agar: (Distilled water 500ml, beef extract 0.5g, year extract 1g, peptone 5g, sodium chloride 2.5g, agar 7.5g)

Washing and Sterilizing of Materials: All the glassware instruments were first washed with the detergent and then they were air dried and then all the material and apparatus were autoclaved This was done to make all the material sterilized so there should be no microbial contamination.

Preparation of Media: The media used (st. NA and st. MacConkey agar) were weighed and prepared according to manufacturer's specification. The prepared media was carefully packed into the autoclave and sterilized at 121°C for 15 minutes. Prior to use, the media were cooled to about 45°C

Isolation and Identification of Bacterial Isolates: After 24 hours of incubation the Bacterial growth were observed on the Petriplates and they were identified with their morphological characteristics, gram reactions and bio chemical characteristics.

Gram Staining Techniques: A thin smear was made by emulsifying a little portion of organisms picked from stocked colony of 18–24 hours old pure culture into a drop of sterile distilled water on a grease free slide. The smear was air dried and heat fixed by passing it slightly over flame. The slide was carefully placed on the staining rack and was flooded with primary stain (crystal violet) for 30–60 seconds. Gram's iodine was added (mordant) for 30 seconds. The smear was gently rinsed with tap water. 70% ethanol was applied as decolouriser for 10–30 seconds; it was the stained with the secondary stain (safranin) for 30 seconds before rinsing with tap water and was allowed to dry. The smear was examined under the microscope using oil immersion objective (x100). Gram positive organisms appeared purple while Gram negative appeared red.

Biochemical Characterization of the Isolates: All These tests were carried out to further identify and classify the culture s. Which include; Catalase test, coagulase test, this test is used to differentiate *Staphylococcus aureus* (positive) from coagulase negative *Staphylococci,* oxidase test,

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Citrate utilization test, motility test, indole Test, urea hydrolysis (urease test) , sugar fermentation test (glucose, sucrose, lactose, galactose, maltose and fructose) respectively.

Oxidase Test: This test is used to identify microorganisms containing the enzyme cytochrome oxidase (important in the electron transport chain). It is commonly used to distinguish between oxidase negative *Enterobacteriaceae* and oxidase positive *Pseudomadaceae*.A piece of filter paper was soaked with a few drops of oxidase reagent (Tetramethyl-p-phenylenediamine dihydrochloride)
A colony of the test organism was then smeared on the soaked filter paper. If the organism could produce oxidase, the phenylenediamine in the reagent will be oxidized to deep purple color. The change of color within 10 seconds indicates positive Result.

Sugar Fermentation test: The carbohydrate fermentation test is used to determine whether or not bacteria can ferment a Specific carbohydrate. Carbohydrate fermentation patterns are useful in differentiating among Bacterial groups or species. It tests for the presence of acid and/or gas produced from carbohydrate fermentation. Basal Medium containing a single carbohydrate source such as glucose, lactose, sucrose or any other Carbohydrate is used for this purpose. A pH indicator bromothymol blue (BTB), is also present in the medium; which will detect the lowering of the pH of the medium due to acid production. Small inverted tubes called durham tube is also immersed in the medium to test for the production of the gas (hydrogen or carbon dioxide). It's a positive test for all members of *Enterobacteriaceae.*

Catalase Test: This test is used to identify organisms that produce the enzyme catalase. This enzyme detoxifies hydrogen peroxide (H2O2) by breaking it down into water and oxygen gas. This test demonstrates the presence of catalase, an enzyme characterized with the release of oxygen from Hydrogen peroxide. A drop of 3% hydrogen peroxide solution was added to the sterile slide containing a loopful of the organism. Foaming or bubble indicates a positive result.

Indole Test: This test is used to identify microbes that can break down tryptophan to indole. It is used to Identify bacteria of the family Enterobacteriaceae. Inoculate sterilized tubes containing tryptophan broth (4 ml) and incubate tubes for 24–28 hrs. After which 0.5 ml of Kovac's reagent is added. Presence/absence of ring indicates positive/negative test.

Citrate Utilization Test: This test is often used to differentiate organisms that are capable of utilizing citrate as a carbon source. Simmon's citrate agar medium was prepared in bijou bottle and allowed to set in a slanting position. A sterile wire loop was used to inoculate the test organism on to the slant medium and incubated at 37˚C for 48 hours after which it was examined for color change. A bright blue color in the medium gave a positive citrate test.
Coagulase Test: Coagulase is an enzyme that clots blood plasma. This test is carried out on Gram positive *Staphylococcus aureus*. A drop of sterile distilled water was placed on each end of a sterile slide. A colony of test organism was emulsified on each spot to make thick suspensions. A loopful

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of plasma was added to one of the suspension and mixed gently. The slide was examined for clumping or clotting of the organism within 10 seconds. Plasma was not added to the second suspension which serves as control.

Urease Test: This is used to identify those organisms that are capable of hydrolysing urea (bacteria that pro-Duce urease) to produce ammonia and carbon dioxide. It is primarily used to distinguish urease positive protease from other *Enterobacteriaceae*. Organisms that hydrolyze urea rapidly (*Proteus spp., Morganella morganii*, and some *Providencia stuartii* strains) will produce strong Positive reactions within 1 or 6 hours of incubation; delayed positive organisms (e.g. Klebsiella Spp and Enterobacter species) will produce weak positive reactions in the slant in 6 hours of Incubation which will be intense during further incubation. The culture medium will remain a Yellowish color if the organism is urease negative e.g. [6] *Escherichia coli.* If organism produces Urease enzyme, the color of the slant changes from light orange to magenta. If organism do not Produce urease the agar slant and butt remain light orange (medium retains original color).[7]

III. RESULT

Bacterial growths were observed after 24 hours of inoculation using the swab sticks, the Observed growth are tabulated below.

Bacterial isolates	No of colonies	Gram character	Shape
Staphylococcus aureus	95	Positive	Cocci
Bacillus spp.	30	Positive	Rod
Coagulase negative Staphylococcus	54	Negative	Spherical
Micrococcus spp.	15	Negative	Spherical
Viridans streptococcus spp.	10	Positive	Cocci
Enterococcus spp.	10	Positive	Cocci
Yeast	20	-	Circular

TABLE 1: Bacteria isolate from keyboard

Bacterial isolates	No of colonies	Gram character	Shape
Pseudomonas	50	Negative	Rod
E. coli	30	Negative	Rod
Serratia	35	Positive	Rod
Micrococcus	45	Positive	Spherical
Salmonella	20	Negative	Rod
Klebsellia	15	Negative	Triangular

TABLE 2: Bacteria isolate from Atm

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Table No. 1 represents, the results which demonstrate the highest positive culture belonged to Bacteria are *Staphylococcus auerus, Coagulase negative bacteria, gram positive bacillus, viridans Streptococcus, enterbaceriaceae. Staphylococcus auerus and enterococcus* were present in high Ratio all over the 5 sample and the plates were also contaminated with saprophytic fungal hyphae and yeast respectively.

Table No. 2 represents, the results which demonstrates the highest Negative culture belonged to Bacteria are *Pseudomonas, E. coli, Salmonella, Klebselli, and* gram positive Bacteria such as, *Serratia, Micrococcus* are the

Main Bacteria associated with the ATM machines.

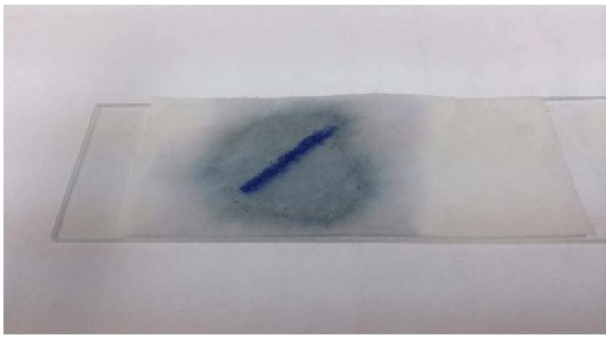
Results for Biochemical characterization of the isolates

3.1 Results for Oxidase Test

(Fig .1)

Positive Oxidase results for organisms

(Fig.2)



Negative Oxidase results for organisms

3.2 Results for Sugar Fermentation Test

Fig.3: Results for sugar fermentation test

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3.3 Results Catalase Test

Fig. 4: Positive Catalase results for organisms.

.4 Results for Indole Test

Fig.5: Positive indole results for organisms.

3.5 Results for Citrate Utilization Test

Fig. 6: Positive citrate utilization test results for organisms.

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3.6 Results for Coagulase Test

Fig.7: Positive coagulase test results for *s.aureus*

3.7 Results for Urease test

Fig.8: Final Urease test for Microorganisms

Table for biochemical test for various organisms

Organism	Oxidase Test	Catalase Test	Indole Test	Citrate Test	Urease Test	Coagulase Test	Sugar fermentation
Staphylococcus aureus	-ve	+ve	-ve	+ve	+ve	+ve	+ve
Bacillus spp.	+ve	+ve	-ve	+ve	-ve	-ve	+ve
Coagulase -ve Staphylococcus	-ve	+ve	-ve	+ve	+ve	-ve	+ve
Micrococcus spp.	-ve	+ve	-ve	-ve	+ve	-ve	+ve
Viridans streptococcus	-ve	-ve	+ve	-ve	-ve	-ve	+ve
Enterococcus spp	-ve	-ve	-ve	-ve	-ve	-ve	+ve
Yeast	-ve	+ve	-ve	-ve	-ve	-ve	+ve
Pseudomonas	+ve	+ve	-ve	+ve	+ve	-ve	-ve
E. coli	-ve	+ve	+ve	-ve	-ve	-ve	+ve
Salmonella	-ve	+ve	-ve	-ve	-ve	-ve	+ve
Klebsellia	-ve	+ve	-ve	+ve	+ve	-ve	+ve

Table 3 for biochemical test for various organisms

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These organisms may probably have found their way into the phone through the skin and from hand to hand. This is because the isolated bacteria are a subset of the normal microbiota of the skin. Staphylococcus aureus is known to cause illness ranging from pimples to boils.[8]

Contamination rate of computer keyboard place were 95% respectively so among all the contaminated keyboard had a cleaning program once a day or once a week to protect us from a harmful infections.

IV. DISCUSSION

1. In this study more than 90% of all computer keyboards and were infected with microorganisms that could contribute to the higher infection.
2. One of the main causes of epidemics obtained from the environment and nosocomial Infection is the bio-contamination of surface of various items and equipment used by the Public.
3. We investigated the occurrence of microorganisms on every day object like computer keyboard the incidence of bacteria was detected on 90% on and maximum bacterial Contamination on the ATM with a mixed flora of gram positive and gram-negative Bacteria and potentially pathogenic or non-pathogenic bacteria.
4. These bacteria under certain circumstances mainly in immunosuppressed person may cause Prevent infection in human.
5. Our studies have confirmed that ATM and computer keyboard is contaminated with more Heterogeneous spectrum of microorganisms like bacteria & fungi as compared to keyboard as evidence by the presence of yeast and fungi that were not detected on phone.
6. Our research demonstrate that microbial contamination of computer keyboard and Atm Is frequent and the most common organism are in skin Commensals.
7. The presence of potentially pathogenic bacteria such as S. aureus, gram positive bacilli and Enteric bacteria represent a risk of infection.
8. The similarity in the bacterial loads recorded on interfaces studied can be attributed To frequent dermal contact and sharing by numerous users with differing hygiene practices and Health conditions.
9. Most skin flora bacteria are Gram- positive, which would account for their predominance on the interfaces.
10. Multiple contamination differs among different occupational And organizations; this could be attributed to differences in hygiene level among these occupational groups and organizational types.
11. This could be Related to the fact that multiple contaminations is influenced by the level of personal hygiene Exhibited by users, since most display a poor level of hygienic practice during interface usage. Multiple contaminations was higher on keyboards and users' hands than on ATMs and mouse Devices; the fact that keyboards are more frequentlyy used than the other interfaces could. Explain the great diversity of bacteria found on them.[9]

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V. CONCLUSION

The study showed that all, ATMs and computer under consideration were infected by several microbes, most of which belonged to natural flora of the human body as well as airborne Fungi and soil. This means that it is necessary to clean our hands after the contact with a phone and Computer keyboard ATMs we should clean a keyboard by once a day or a week since it is a source of disease transmission. We can reduce the presences of microorganisms on device like computer Keyboard and ATMs by using commercially available antibacterial wet wipes. It helps to the reduction of Bacterial contamination after the disinfectant achieved even 100% in the case of enteric bacteria n Atm and also 100% in case of Staphylococcus aureus or Streptococcus spp. On the Surface of computer keyboard after the disinfection no yeast or moulds were present on the devices

The banks themselves should be held responsible for frequent cleaning of these devices as users are actually patronizing the banks and some increasing their profit margins. Anyway, the need for hand-sanitizers and hand wash points Next to the ATM machines is an urgent necessity that should be implemented in the near future, An issue that deserves to be raised to the decision makers for better quality of life in our society.[10]

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