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Isolation and Characterization of Microbial Contamination from Computer Accessories used in Different Department of Hazara University and Diagnostic Laboratories of District Mansehra, Pakistan

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Abstract 150 samples were collected from computer accessories used in Hazara University and different diagnostic laboratories of Mansehra, examined for the total bacterial count and maximum growths were observed. Samples were analyzed for further identification of micro-organisms such as *E.coli*, *Klebsiella*, *Staph. aureus* and *Staph. epidermidis.* These organisms were detected in the percentage of 46.66 of *E. coli*, 20% of *Klebsiella*, 16.66% of *S. aureus* & 16.66% of *S. Epidermidis* and identified on selective media, i.e. EMB agar and Mannitol salt agar. Furthermore, biochemical tests, including IMVIC Test, Catalase Test and Coagulase Tests were performed to confirm the presence of micro-organisms and their susceptibility also checked against different standard antibiotics and their zone of inhibitions were measured and noticed. *E.coli* showed maximum resistance of 97.36% against Erythromycin, *Klebsiella* showed against Amoxil + Clavolunic acid about 83.83%, *Staph aureus* showed against Erythromycin about 64.64% and *Staph. epidermidis* resistance was 90.9% against Erythromycin and Gentamycin. *E.coli* and *Klebsiella* showed maximum sensitivity for Meropenem 67.22% and 72.72% respectively while *Staph. aureus* and *Staph. epidermidis* maximum sensitivity for Vancomycin about 82.82% and 72.72% respectively. These results indicate that the computer accessories might act as environmental vehicles for the transmission of potentially pathogenic bacteria in our surroundings and also indicate the need for increasing awareness among computer users on cleaning of such surfaces or disinfection and adequate hand-washing hygiene.

Keywords: *E.coli, Klebsiella, Staph. aureus, Staph. epidermidis, Computer accessories, EMB agar, Mannitol salt agar, Antibiotic resistance*

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1. Introduction

The skin floras include 1000 different species of bacteria belonging to 19 different phyla have been isolated from human skin, are usually non-pathogenic either commensal or mutualistic (Bhalla *et al*., 2007). Bacteria prevents pathogenic organisms from colonizing on the skin surface, either by competing for nutrients or secreting chemicals against them and by stimulating skin's immune system as well (Grice *et al.,* 2009; Oluduro *et al.,* 2011). However, microbes cause skin diseases via penetrating into the blood stream and fabricate life-threatening diseases, particularly in people with weak immune system (Cogen *et al.,* 2008). E.g. *Pseudomonas aeruginosa*, a pathogenic mutualist bacterium, causes gastro, respiratory, osteo and skin infections via blood but it produces a substances that inhibits the growth of fungus species like *Candida krusei*, *Candida albicans*, *Torulopsis glabrata*, *Saccharomyces cerevisiae* & *Aspergillus fumigatus* and bacteria like *Helicobacter pylori* (Krausse *et al.,* 2005). The skin flora strengthens the person's immunity.

Computers are widely used in every aspect of our occupational, recreational and residential environments. In the university setting, students have 100% access to computers, 92.1% regularly use the Internet and 73.3% regularly check e-mail (Ali *et al.,* 2013). A research

conducted in a hospital ICU revealed that computer keyboards and door knobs had higher rates of bacterial colonization with *methicillin-resistant S.aureus (MRSA)* and other potential nosocomial pathogens. They become responsible for transmission of microbes in the ICU setting (Bures *et al.*, 2000). In another study in 2006, it has been reported that potential pathogens, including Coagulase-negative *Staphylococci, Diphtheroids, Micrococcus species, Bacillus* species, *Staphylococcus aureus, Enterococcus species* and non fermentative gram-negative rods were found in 25 computer keyboards in two intensive care units and six nursing units (Rutala *et al.,* 2006). Coagulase-Negative *Staphylococcus aureus,* usually found on the skin or in the nasal areas. It appears on keyboards while usage and responsible to cause infections in hospital community (David and Daum, 2010). So, the computer keyboards and mouse may act as a reservoir for the transmission of pathogenic bacteria.

That's why; Computer accessories of multiple-user (student) and single-user (staff) in Hazara University, Mansehra campus was sampled to assess microbial contamination with potentially pathogenic microorganisms. This study was aimed to investigate the extent of microbial infection, which could affect human health through using computer accessories.

2. Materials and Methods

A one year study was conducted in the Microbiology Department of Hazara University, Mansehra, during January to December 2010. Total 150 samples were collected from computer keyboards and mouse by sterile swabs from different Departments, Digital libraries of Hazara University and different Diagnostic Laboratories of District Mansehra. In this study bacterial pathogens were isolated and their antimicrobial susceptibility was studied (Forbes *et al.,* 2007).

a. **Sampling Sites and their Collections:** Samples were collected from the computer hardware's of different location divided into three groups.
   - **Group A:** 50 samples were taken from different departments and offices of Hazara University Mansehra.
   - **Group B:** Another 50 samples were collected from computer labs of Hazara University, Mansehra.
   - **Group C:** 50 other samples were collected from different diagnostic laboratories of Mansehra.

b. **Sterilization of the Glassware:** All the glassware and equipments such as test tubes, petri dishes, spreader, wire loops, beakers and flasks were sterilized in an autoclave at 121°C and 15psi pressure for 15 min. Before sterilization, glass wares were wrapped in the aluminum foil to prevent contamination.

c. **Media Preparation:** 500ml distilled water was taken in flask, for boiling placed on hot plate, then added 18.45gm of media in the flask (Table1). Shook the mixture until homogenization. That homogenized media was autoclaved to eliminate contamination. Now media were poured in sterile petri dishes inside the laminar flow hood.

d. **Identification of bacterial isolates:** The bacteria were isolated on autoclaved media. The media after cooling down were aseptically poured in the test tubes and petri dishes under laminar flow hood. The specimens were spread by using a sterilized wire loop on nutrient agar. After 24 hours incubation at 37°C in aerobic conditions, these plates were examined for any growth. Furthermore the positive growth plates were sub cultured on specific media such as EMB and MSA agar. Isolated colonies of bacteria were identified by morphological appearance and different biochemical tests (Koneman, 2006; Sahara *et al.,* 2011). The following tests were performed for the identification of bacteria:

1. Indole Production Test
2. Methyl Red Voges-Proskauer (MRVP) Test
3. Citrate Utilization Test
4. Catalase Test
5. Coagulase Test

Table 1. Culture Medias and their Composition

Media	Ingredients	Amount (gm/L)
Nutrient Agar pH 7.4± 0.2 at 25°C	Lab-Lamco Powder	1.0
	Yeast Extract	2.0
	Peptone	5.0
	Sodium Chloride	5.0
	Agar	15.0
Mannitol Salt Agar pH 7.4± 0.2 at 25°C	Lab-Lamco Powder	1.0
	Peptone	10.0
	Mannitol	10.0
	Sodium Chloride	75.0
	Phenol Red	0.025
	Agar	15.0
Muller Hinton Agar	Meat Infusion	6.0
	Casein Hydrolyte	17.5
	Starch Agar No.1	1.510.0
Eosin methylene blue (EMB) pH7.1±o.2 at 25°C	Pepton	10.0
	Di-potassium hydrogen phosphate	2.00
	Lactose	5
	Sucrose	5
	Eosin yellowish	0.4
	Methylene blue	0.07
	Agar-agar	13.5
Tryptone Water pH 7.3± 0.2 at 25°C	Tryptone	10.0
	Sodium Chloride	5.0

1: Indole Test: Test organism inoculates into trypton broth, rich source of amino acid tryptophan. Indole positive bacteria such as *E. coli* produces tryptophans, an enzyme that cleaves tryptophan, produces Indole and other products. When Kovac's reagent (P-dimethylaminobenzaldehyde) adds to a broth with Indole, a dark pink color develops. The Indole test must be read by 48hrs of incubation because the insole can be further degraded if prolong the incubation occurs. The acidic pH produced by *E. coli* limits its growth (Winn *et al.,* 2006).

2: Methyl red and Voges-Proskauer Test: After 24-48hrs of incubation the MR-VP broth splits into two tubes. One tube is used for the MR test and the other for VP test. MR-VP media contain glucose and peptone. All enteric bacteria oxidize glucose for energy. *E. coli* is one of the bacteria that produce acids and causing the pH to drop below 4.4. When an acid indicator methyl red is added to this acidic broth it give cherry red color resulting in MR positive test. *Klebsiella* and *Enterobacter* produce more neutral products from glucose. In this neutral pH the growth of the bacteria does not inhibit. These bacteria begin to attack the peptone in the broth, causing the pH to rise above 6.2. At this pH, the methyl red indicator is a yellow color resulting in MR negative test. The reagents

used for the VP test are Barrett's reagents and when Barrett's reagents are added to a broth it gives pink-burgundy color resulting in positive VP test. This color may take 20-30min to develop.

3: Citrate Test: This test utilizes Simon's citrate media to determine if a bacterium can grow utilizing citrate as its sole carbon and energy source. Simon's media contain bromothymol blue, a pH indicator with a range of 6.0 to 7.6. Bromothymol blue is yellow at acidic pH (around 6) and gradually changes to blue at more alkaline pH (around 7.6). Un inoculated Simon's citrate agar has pH of 6.9. So it's an intermediate green color. Growth of bacteria in the media leads to the development of a blue color (positive citrate). *Enterobacter* and *Klebsiella* are citrate positive while *E.coli* is negative. Thus *E.coli* + + - - results on the IMVIC tests, while *Enterobacter* and *Klebsiella* give the reverse - - + +.

4: Catalase Test: The presence of catalase enzyme in the test isolate is detected by using of H_2O_2. When a small amount of bacterial isolate is added to hydrogen peroxide, bubbles of oxygen or froth produce and show catalase positive result. This test is used to differentiate between different bacterial species in the lab. It is done by placing a drop of H_2O_2 on a microscope slide by using an applicator stick, touches the colony and smears a sample into H_2O_2. *Staphylococci* and *Micrococcus* are catalase-positive, but *Streptococci* and *Enterococci* are catalase-negative.

5: Coagulase Test: The enzyme coagulase causes plasma to clot by converting fibrinogen to fibrin and organisms to agglutinate in small quantities of plasma. The test is performed by adding some blood plasma to a drop of normal saline on a slide by using an applicator stick touch the colony and smears a sample into the diluted plasma. Agglutination indicates that the test is coagulase positive (Qian *et al.,* 2007).

e. Antimicrobial susceptibility Test: The antimicrobial susceptibility test or disc diffusion method is used for each bacterial isolate on Mueller Hinton agar as a growth medium. 25 ml of media is poured in 90 mm sterile petri dishes and incubate at 37°C overnight to check sterility.

f. Inoculation preparation: Tryptic Soya broth is made for inoculum preparation. 5ml of broth medium is dispensed in test tubes and sterilized by autoclaving at 121°C for 15 mints. The test tubes are cooled and kept in an incubator for 24 hours at 35°C to check the sterility. Then each of identified clinical isolate is inoculated in sterilized test tubes containing media and placed in incubator for 2-6 hrs at 35°C.

3. Results

a. Sample collection:

It was observed that out of 50 samples from group "A" numerous, or uncountable bacterial colonies were found in most of the samples except sample No. 4,6-8, 10, 12, 15, 17, 19, 20, 23-25, 28, 30, 33, 34, 37-39, 41, 44, 46-47, 49-50 (Table 2). Out of 50 Samples collected from group "B", uncountable bacterial colonies were found in most of the samples except sample No.1, 3, 6, 8, 11, 13, 16, 21, 27, 30, 34, 37, 39, 41, 43, 49, 50 (Table 3). Out of 50 samples collected from group "C", numerous uncountable bacterial colonies were found in most of the samples except sample No. 1, 3, 6, 9-10, 12, 16, 21, 29, 32, 35, 37, 39, 43, 48 (Table 4).

Table 2. Bacterial counts in samples collected from different Departments of University (Group "A")

S. No	Sample Code	No of Bacterial Count/ml.	Value in Log.	S. No	Sample Code	No of Bacterial Count/ml.	Value in Log.
1.	Law.Ch.M	82×10^5	6.91	26	M.M	Uncountable	-
2.	Law.L.M	Uncountable	-	27	M.K	Uncountable	-
3.	Law.Z.M	Uncountable	7.04	28	M.Z.M	136×10^5	7.13
4	Bio.HOD.K	65×10^4	5.81	29	M.J.M	Uncountable	_
5	Bio.L.M	Uncountable	_	30	M. Sh.K	75×10^4	5.87
6	Bio. M	90×10^4	5.95	31	M.Ds.K	Uncountable	_
7	EngCh.K	55×10^4	5.74	32	M.Ch.K	Uncountable	_
8	Eng.K.	35×10^3	4.54	33	Gen.M	147×10^4	6.16
9	Eng.L.K	Uncountable	-	34	Gen.K	202×10^2	4.30
10	Edu.HODM	20×10^2	3.36	35	Gen. L	Uncountable	_
11	Edu.Lab.K	Uncountable	_	36	Mt.M	Uncountable	_
12	Edu.M	75×10^4	5.87	37	Mt.K	167×10^4	6.22
13	Edu.K	Uncountable	_	38	Mt.HOD.K	75×10^2	3.87
14	IT.Ch.M	Uncountable	_	39	Mt.M	98×10^2	3.99
15	IT.Lab1.M	155×10^3	5.19	40	PS.M	Uncountale	_
16	IT.Lab2.K	Uncountable	_	41	PS.K1	112×10^5	5.05
17	Z. HOD.K	80×10^4	5.90	42	PS.K2	Uncountable	_
18	Z.Lab.K	Uncountable	_	43	PS.L1.M	Uncountable	_
19	Ph. HOD.K	82×10^5	6.91	44	FA.M	Uncountable	-
20	Ph. L1.K	110×10^5	7.04	45	FA.K	Uncountable	-
21	Ph. L2.K	Uncountable	_	46	FA.Ch.M	134×10^5	5.1
22	Ph. L3.K	Uncountable	-	47	HPE.1	166×0^5	5.22
23	Ph.S.M	112×10^5	5.05	48	HPE.K1	Uncountable	-
24	Bot.L.M	185×10^4	6.26	49	HPE.K2	175×10^4	6.24
25	Bot.L.K.	198×10^4	6.23	50	HPE.2	188×10^4	6.27

Total 50 samples were collected from different Departments (group "A") of Hazara University out of which 23 were uncountable or numerous and 27 were countable.

Table 3. Bacterial counts in samples collected from digital libraries of university (Group "B")

S. No	Sample Code	No of Bacterial Count/ml.	Value in Log.	S. No	Sample Code	No of Bacterial Count/ml.	Value in Log.
1	Mi.DL.M 1.	230×10^5	7.36	26	Ge.DL.K2.	Uncountable	_
2	Mi.DL.M 2.	Uncountable	_	27	Ge.DL.K3.	241×10^4	6.38
3	Mi.DL.K1.	225×10^3	5.35	28	IT.DL.M1.	Uncountable	_
4	Mi.DL.K2.	Uncountable	_	29	IT.DL.M2.	Uncountable	_
5	Mi.DL.K3.	Uncountable	_	30	IT.DL.M3.	230×10^5	7.36
6	Mi.DL.M2.	235×10^5	7.40	31	IT.DL.M4.	Uncountable	_
7	Ge. DL.M1.	Uncountable	_	32	IT.DL.M5.	Uncountable	_
8	Ge.DL.M2.	188×10^4	6.27	33	IT.D.M6.	Uncountable	_
9	Ge.DL.M3.	Uncountable	_	34	IT.DL.K1.	237×10^5	7.37
10	Ge.DL.K1.	Uncountable	_	35	IT.DL.K2.	Uncountable	_
11	Edu.DL.M1.	205×10^5	7.31	36	IT.DL.K3.	Uncountable	_
12	Edu.DLM 2.	Uncountable	_	37	IT.DL.K4.	215×10^5	7.33
13	Edu.DL.M3.	198×10^5	7.29	38	IT.DL.K5.	Uncountable	_
14	Edu.DL.M4.	Uncountable	_	39	IT.DL.K6.	225×10^5	7.35
15	Edu.DL.K1.	Uncountable	_	40	Edu.DL.M3.	Uncountable	_
16	Edu.DL.K2.	213×10^5	7.32	41	Edu.DL.M4.	235×10^5	7.37
17	Edu.DL.K3.	Uncountable	_	42	Ph.DL.M1.	Uncountable	_
18	Edu.DL.K4.	Uncountable	_	43	Ph.DL.M2.	220×10^5	7.34
19	Edu.DL.M5.	Uncountable	_	44	Ph.DL.K1.	Uncountable	_
20	Edu.DL.M6.	Uncountable	_	45	Ph.DL.K2	Uncountable	_
21	Edu.DL.K5.	233×10^5	7.36	46	Ph.DL.K3.	Uncountable	_
22	Eng.DL.M1.	Uncountable	_	47	Ph.DL.K4.	Uncountable	_
23	Eng.DL.M2.	Uncountable	_	48	Ph.DL.K5.	Uncountable	_
24	Eng.DL.K1.	Uncountable	_	49	Ph.DL.M3.	220×10^5	7.34
25	Eng.DL.K2.	Uncountable	_	50	Ph.DL.M4.	220×10^5	7.34

Total 50 samples were collected from different Digital labs (group "B") of Hazara University out of which 33 were uncountable or numerous and 17 were countable.

Table 4. Bacterial counts in samples collected from different diagnostic laboratories of Mansehra (Group "C")

S. No	Sample Code	No of Bacterial Count/ml.	Value in Log.	S. No	Sample Code	No of Bacterial Count/ml.	Value in Log.
1	AL.DL.M1.	240×10^5	7.38	26	MA.DL.K3.	Uncountable	_
2	AL.DL.M2.	Uncountable	_	27	MA.DL.M3.	Uncountable	_
3	Al.DL.K1.	225×10^4	6.35	28	MA.DL.M4.	Uncountable	_
4	Al.DL.K2.	Uncountable	_	29	MA.DLM 5.	230×10^5	7.36
5	Al.DL.K3.	Uncountable	_	30	MA.DL.M6.	Uncountable	_
6	Al.DL.M3.	235×10^5	7.40	31	MA.DL.K3.	Uncountable	_
7	l.DL.M4.	Uncountable	_	32	MA.DL.K4.	237×10^5	7.37
8	Al.DL.K4.	Uncountable	_	33	MA.DL.K5.	Uncountable	_
9	Al.DL.M5.	205×10^5	7.31	34	MA.DL.K6.	Uncountable	_
10	Al.DLK5.	198×10^5	7.29	35	NA.DL.K1.	225×10^5	7.35
11	MA.DL.K1.	Uncountable	_	36	NA.DL.M1.	Uncountable	_
12	MA.DL.M1.	199×10^5	7.29	37	NA.DL.M2.	235×10^5	7.37
13	MA.DL.M2.	Uncountable	_	38	NA.DL.M3.	Uncountable	_
14	ST.DL.M1.	Uncountable	_	39	NA.DL.M4.	220×10^5	7.34
15	ST.DL.M2.	Uncountable	_	40	NA.D L.K2.	Uncountable	_
16	ST.DL.M3.	238×10^5	7.37	41	NA.DL.K3.	Uncountable	_
17	ST.DL.M4.	Uncountable	_	42	NA.DL.K4.	Uncountable	_
18	ST.DL.M5.	Uncountable	_	43	NA.DL.K5.	234×10^4	6.37
19	ST.DL.K1.	Uncountable	_	44	NA.DL.M5.	Uncountable	_
20	ST.DL.K2.	Uncountable	_	45	NA.DL.M6.	Uncountable	_
21	ST.DL.K3.	215×10^5	7.33	46	NA.DL.M7.	Uncountable	_
22	ST.DL.K4.	Uncountable	_	47	ST.DL.M2	Uncountable	_
23	ST.DL.K5.	Uncountable	_	48	ST.DL.M3.	200×10^5	7.30
24	MA.DL.K2.	Uncountable	_	49	ST.DL.M4.	Uncountable	_
25	MA.DL.K2.	Uncountable	_	50	ST.DL.M5.	Uncountable	_

Total 50 samples were collected from different Digital labs (group "C") of Hazara University out of which 35 were uncountable or numerous and 15 were countable.

b. **Samples Dilutions:** To know the numbers of bacterial colonies in each sample serial dilutions were prepared for up to seven dilutions to give countable growth on nutrient agar (Figure 1).

c. **Identification and characterization of Microorganisms found in the samples:** Different samples were collected from computer accessories by sterile swabs and inoculated on nutrient agar by spreading method. The numbers of colonies were determined with the help of the colony counter (Figure 2).

Figure 1. Samples dilution test

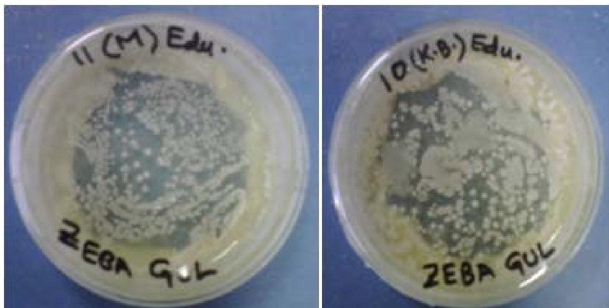

Figure 2. Growth of microorganisms on nutrient agar

d. **Identification using selective media:** The bacterial cultures grown on nutrient agar were further inoculated on selective media.

e. **Growth on EMB Agar:** The Eosin methylene blue (EMB) agar was used for isolation of enteric gram negative lactose ferments. In some of samples green metallic sheen was appeared on EMB media, which had shown the presence of *E. coli* (Figure 3).

f. **Mannitol Salt Agar:** For further confirmations another selective media Mannitol salt agar was used. The appearance of yellow colonies confirmed the presence of *S. aurous* (Figure 4).

Figure 3. *E. Cole* raised colonies with a green metallic sheen on EMB agar

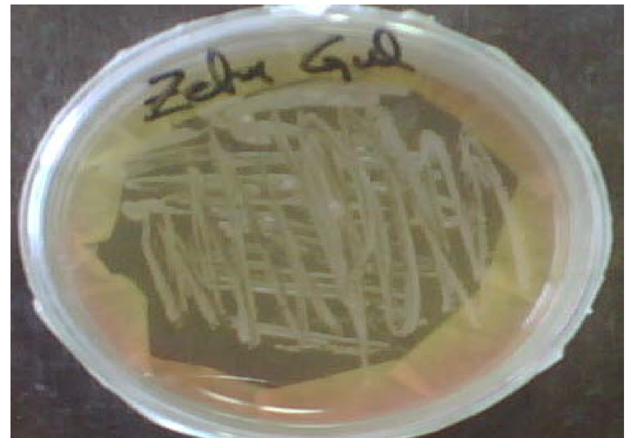

Figure 4. Growth of Staphylococcal *species* on Mannitol salt agar

g. **Catalase Test:** The colonies from EMB medium and Mannitol salt agar medium were picked and placed on a slide. A drop of hydrogen peroxide was put with the help of the wire loop and formation of bubble indicated the positive result. *E. Coli, Klebsiella, S. aureus* and *S. epidermidis* showed catalase positive (Figure 5).

Figure 5. Catalase positive test

h. **IMVIC Test:** These biochemical tests were performed only for G -ve bacteria. These tests were conducted to confirm E.coli, *Klebsiella* etc. Details of these tests are as under;

- **Indole Test:** Trypton broth was prepared, picked the colony from EMB media, put in the broth and incubated for 48hrs at 37°C. After 48hrs Kovac's reagent was put in it which gave a dark pink ring resulting in a positive test (Figure 6).

Figure 6. Indole Negative test and Indole Positive test

- **Methyl red test:** Methyl Red (MR) and Vogues proskaur (VP) were added to the Trypton Water broth for bacterial growth. The broth was separated into two different tubes, one for the addition of MR and other for VP. After 24hrs of incubation, 2-3 drops of Methyl red were put in one test tube. It gave cherry red color it indicates a positive result and yellow color in case of negative result. *E coli* showed methyl red positive and *Klebsiella* methyl red negative (Figure 7).

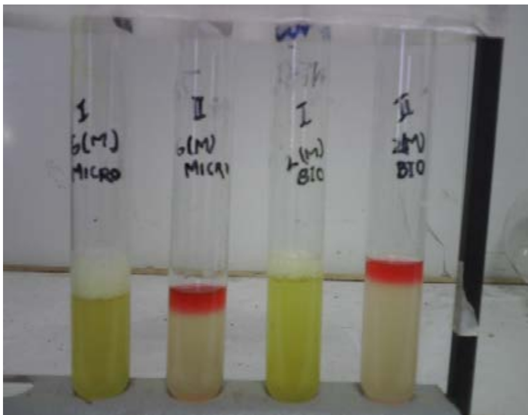

Figure 7. Methyl red Negative test and Methyl red Positive test

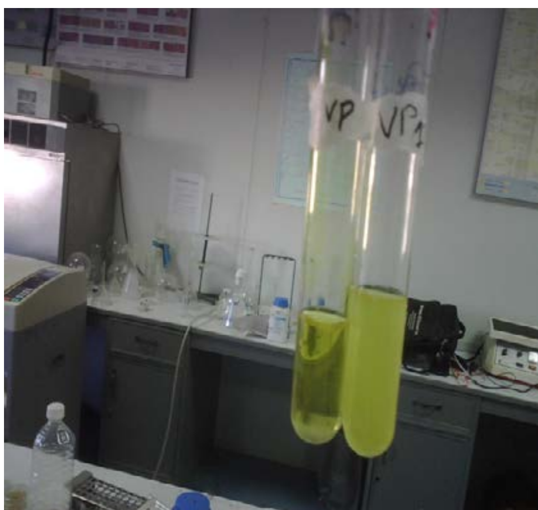

Figure 8. Vogues proskaur Negative test (*E.coli*)

- **Vogues proskaur:** After 24hrs of incubation Barrett's reagent was added to a broth. It remained yellow in case of negative results and it changed into pink color for positive results. *E.coli* was found to be VP negative and *Klebsiella* VP positive (Figure 8).
- **Simmons's citrate:** media were prepared and put in test tubes. The colonies from EMB media were picked with the help of the wire loop and streaked. Blue color indicated the positive result and no color change for the negative result. *E.coli* was citrate negative and *Klebsiella* citrate was positive (Figure 9).

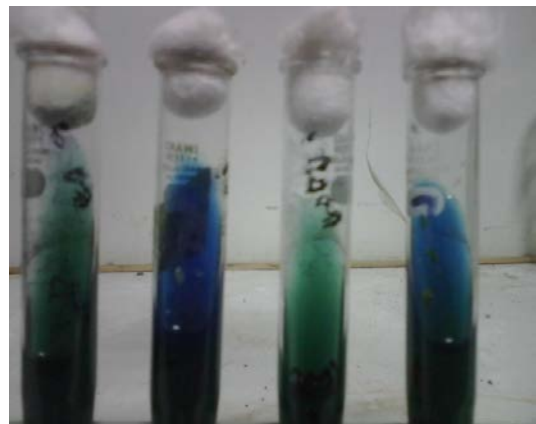

Figure 9. Simmon citrate blue positive test (*Klebsiella)* and negative test (*E. coli)*

Figure 10. Coagulase positive test (*S.aureus)*

- **Coagulase Test:** Blood was centrifuged and one drop of plasma was placed on a slide. The colonies

were picked from Mannitol salt agar and put on the slide. Agglutination indicated the positive result. *S. aureus* was coagulase positive (Figure 10).

Table 5. Biochemical tests for identification of *E.coli*

Specie	Indole Production test	Methyl red Test	Voges Proskaur test	Citrate Utilization test	Catalase Test
E.coli	+ive	+ive	-ive	-ive	+ive

The following 70 samples were *E.coli* positive.

Out 0f 50 samples from group "A", 28 samples were *E.coli* Negative except samples No.1, 3, 4, 5, 7, 8, 10, 13, 19, 22, 26, 27, 30, 31, 33, 34, 36, 37, 40, 45, 49 (Table 2). Out of 50 samples from group "B", 25 samples were *E.coli* Negative except samples No.2, 3, 6, 7, 8, 9, 11-13, 15, 16, 19, 22, 24, 25, 27, 28, 31, 35, 37, 40, 44, 45, 48, 50 (Table 3). Out of 50 samples from group "C", 23 samples were *E.coli* Negative except samples No.1, 3, 7, 8, 10,14-16, 19, 21, 25-27, 33, 35, 36, 38, 39, 41-44, 48 (Table 4).

Table 6. Biochemical tests for the occurrence of *Klebsiella*

Species	Indole Production test	Methyl red Test	Voges Proskaur test	Citrate Utilization test	Catalase Test
Klebsiella	-ive	-ive	+ive	+ive	+ive

The following 30 samples are *Klebsiella* positive.

Out of 50 samples from group "A" most of the samples are *Klebsiella* Negative except samples No.2, 6, 9, 11, 12, 21, 28, 29, 32, 35, 38, 42 (Table 2). Out of 50 samples from group "B" most of the samples are *Klebsiella* Negative except samples No. 1, 10, 14, 26, 29, 30, 32, 34, 42, 43 (Table 3). Out of 50 samples from group "C" most of the samples are *Klebsiella* Negative except samples No. 6, 22, 24, 28, 31, 32, 40, 49 (Table 4).

Table 7. Biochemical tests for the occurrence of *S.aureus*

Specie	Catalase Test	Coagulase Test
S. aureus	+ive	+ive

The following 25 samples are *S. aureus* positive.

Out of 50 samples from group "A" 42 samples were *S. aureus* Negative except samples No. 14, 16, 17, 25, 39, 43, 46, 50 (Table 2). Out of 50 samples 42 samples were *S. aureus* Negative except samples No. 17, 20, 23, 33, 36, 39, 41, 49 (Table 3). Out of 50 samples 41 samples were *S. aureus* Negative except samples No. 2, 4, 5, 9, 17, 20, 23, 29, 30 (Table 4).

Table 8. Biochemical tests for the occurrence of *S. epidermidis*

Specie	Catalase Test	Coagulase Test
Staph. epidermidis	+ive	-ive

The following 25 samples are *S. epidermidis* positive.

Out of 50 samples from group "A" most of the samples were *S. epidermidis* Negative except samples No.15, 18, 20, 23, 41, 44, 48 (Table 2). Out of 50 samples from group "B" most of the samples were *S. epidermidis* Negative except samples No. 4, 5, 18, 21, 38, 46, 47 (Table 3). Out of 50 samples from group "C" most of the samples were *S. epidermidis* Negative except samples No. 11-13, 18, 20, 34, 37, 45-47, 50 (Table 4).

Table 9. Number of samples isolated from different specimens

S. #	Samples	No of Isolates	Percentage
01.	Keyboard	74	49.33
02.	Mouse	76	50.66
Total		**150**	**100**

Table 10. Number of Bacteria isolated during the study (n=150)

S. No.	Isolated Bacteria	Quantity	Percentage
Gram Negative			
1	*E.coli*	70	46.66
2	*Klebsiella spp.*	30	20
Gram Positive			
3	*Staph. aureus*	25	16.66
4	*Staph. epidermidis*	25	16.66
Total		**150**	**99.986**

Antibiotics Sentivity

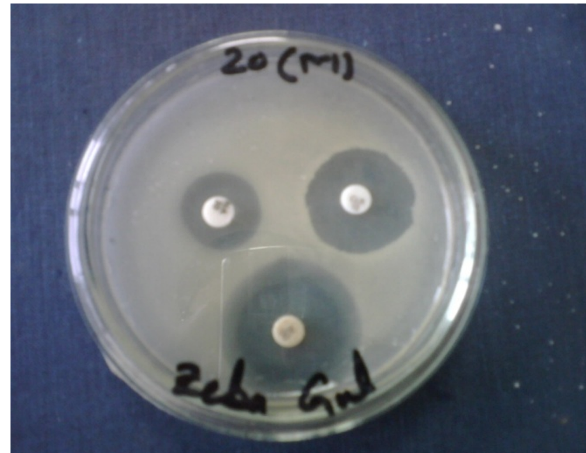

Figure 11. Antibiotics made zone

4. Sensitivity of Antibiotics

Sensitivity of antibiotics against different microbes and the resistance of these microbes against antibiotic were used.

- **Escherichia coli:**

The activity of Meropenem was maximal against *E.coli.* All other antibiotics have activity in the decreasing order of Sulbactum + Cefoperazone > Piperacillin + Tazobactam > Doxycycline, Gentamycin > Ciprofloxacin > Ceftizoxime > Amoxicillin > Amoxil + Clavulanic acid, Sulphamethizole + Trimethoprim > Vancomycin > Erythromycin, Cephradine. The resistance of *E.coli* showed against Erythromycin was maximal. The other drug resistance was in the decreasing order of Vancomycin, Amoxil + Clavulanic acid > Sulphamethizole + Trimethoprim > Cephradine > Ciprofloxacin > Gentamycin > Doxycycline > Ceftizoxime > Piperacillin + Tazobactam > Sulbactum + Cefoperazone > Meropenem shown in Table 11, Figure 12.

- **Klebsiella:**

The activity of Meropenem was maximal against *Klebsiella*. All other antibiotics have activity in decreasing order of Piperacillin + Tazobactam > Ciprofloxacin, Sulbactum + Cefoperazone > Gentamycin, Doxycycline > Amoxil + Clavulanic acid, Amoxicillin > Sulphamethizole + Trimethoprim > Ceftizoxime > Vancomycin > Cephradine > Erythromycin. The resistance of *Klebsiella* showed against Amoxil + Clavulanic acid was maximal. Other drugs resistance was in the decreasing order of Amoxicillin, Sulphamethizole + Trimethoprim > Cephradine, Ceftizoxime > Gentamycin > Ciprofloxacin > Vancomycin, Doxycycline > Erythromycin > Sulbactum +

Cefoperazone > Piperacillin + Tazobactam > Meropenem (Table 12, Figure 13).

Table 11. Antibiotics Sensitivity against *E. coli* strains in Percentage

Sensitivity Pattern	AMOXIL, CLAVULANICACID	AMOXICILLIN	CEPHRADINE	CEFACLOR	CEFTIZOXIME	CIPROFLOXACIN	ENOXACIN	ERYTHROMYCIN	MEROPENEM	GENTAMYCIN	DOXICYCILIN	VANCOMYCIN	SULPHAMETHAXAZOLE, TRIMRTHOPRIM	SULBACTUM, CEFOPERAZONE	PIPRACILLIN, TAZOBACTUM
Sensitive	18.19	22.24	3.3	23.68	27.8	27.1	41.41	2.63	**67.22**	42.93	39.47	7.89	14.15	63.15	52.63
Intermediate	0	0	26	10.52	27.8	7.25	12.12	0	26.1	6.75	13.15	5.26	8.35	21.05	28.94
Resistant	81.81	77.76	70.7	65.78	44.4	65.65	53.53	**97.36**	6.68	50.5	47.36	86.84	77.5	15.78	18.42

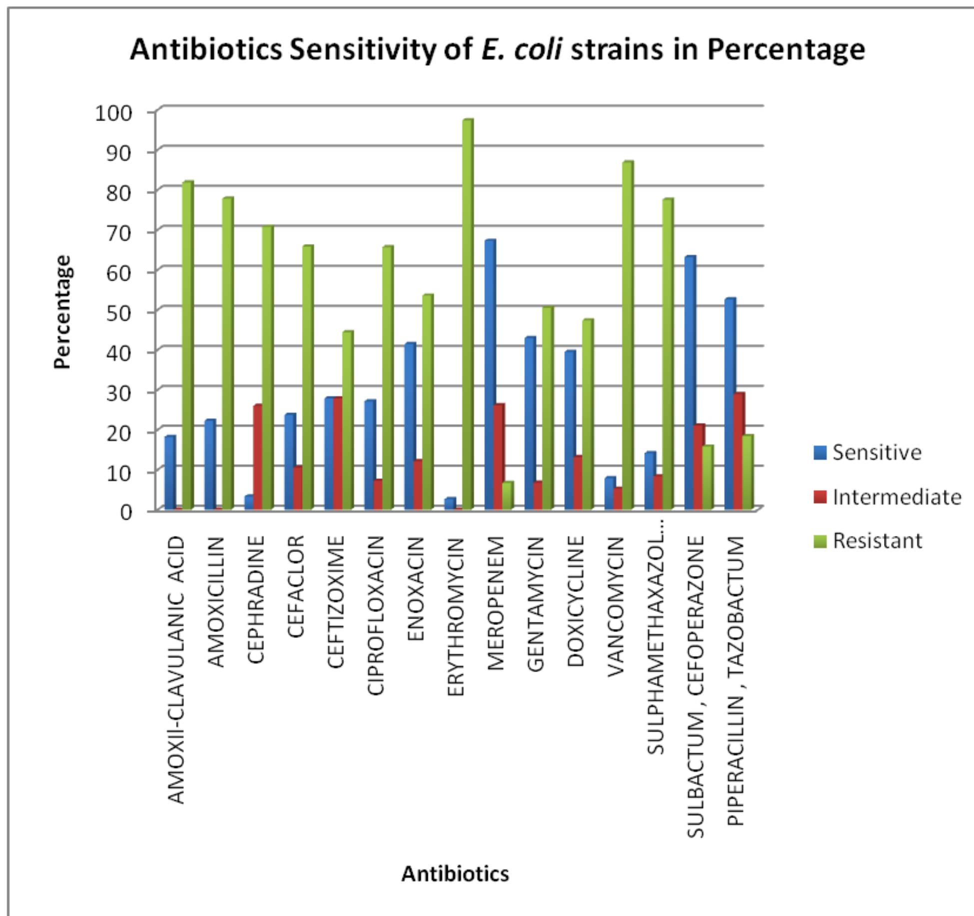

Figure 12. Antibiotics Sensitivity of *E. coli* strains in Percentage

Table 12. Antibiotics Sensitivity against *Klebsiella* strains in Percentage

Sensitivity Pattern	AMOXIL, CLAVULANICACID	AMOXICILLIN	CEPHRADINE	CEFACLOR	CEFTIZOXIME	CIPROFLOXACIN	ENOXACIN	ERYTHROMYCIN	MEROPENEM	GENTAMYCIN	DOXICYCILIN	VANCOMYCIN	SULPHAMETHAXAZOLE, TRIMRTHOPRIM	SULBACTUM, CEFOPERAZONE	PIPRACILLIN, TAZOBACTUM
Sensitive	16.17	21.05	3.25	21.21	3.25	42.74	42.1	57.58	72.72	30.3	39.47	39.47	13.15	63.15	70.7
Intermediate	0	0	25.04	14.15	25.04	8.78	5.26	0	22.88	8.09	13.15	13.15	7.89	21.05	19.19
Resistant	**83.83**	78.94	71.71	64.64	71.71	48.48	52.63	42.42	4.4	61.61	47.36	47.36	78.94	15.78	10.81

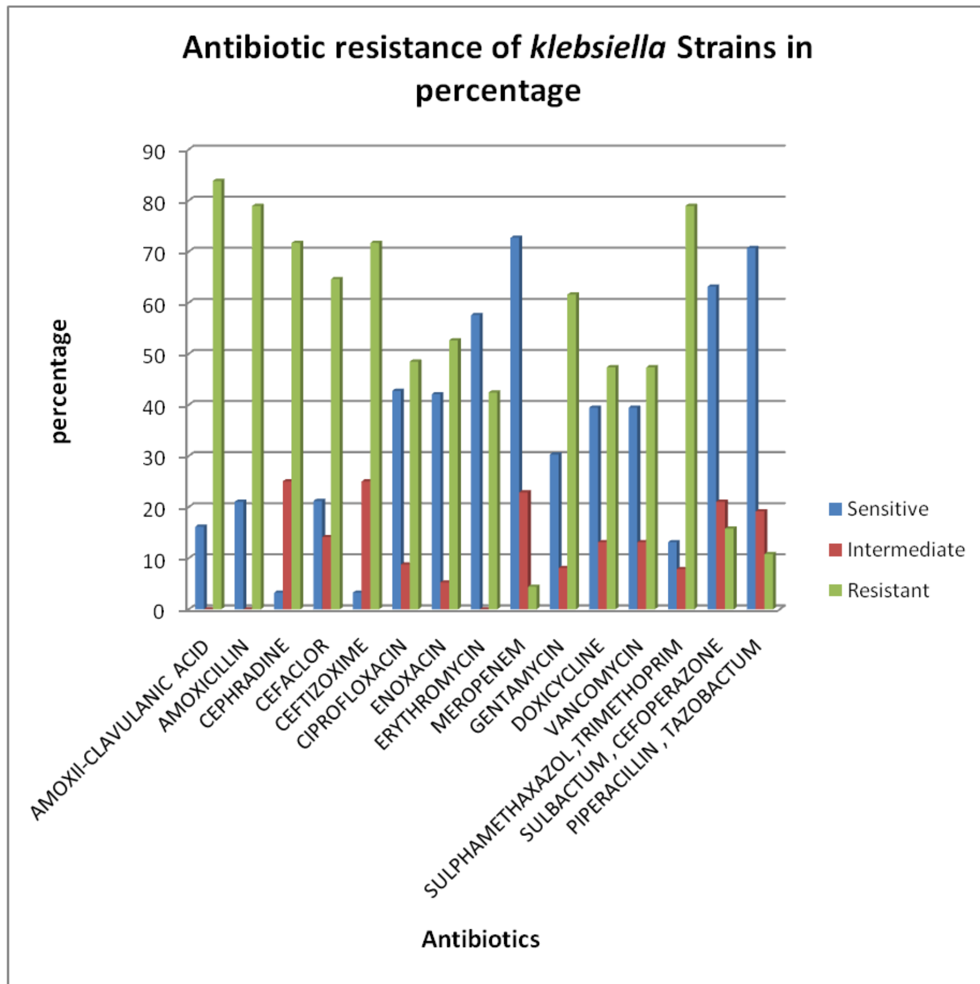

Figure 13. Antibiotics Sensitivity of *Klebsiella* strains in Percentage

- **Staph. Aureus**

The activity of Vancomycin was maximal against *Staph .aureus*. All other antibiotics have activity in decreasing order of Doxycycline > Amoxicillin > Sulbactum + Cefoperazone > Gentamycin, Meropenem > Ciprofloxacin, Piperacillin + Tazobactam > Cephradine > Amoxil + Clavulanic acid > Erythromycin > Ceftizoxime > Sulphamethizole + Trimethoprim. The resistance of *Staph. aureus against* Gentamycin was maximal. The other drug resistance was in the decreasing order of Cephradine > Sulphamethizole + Trimethoprim > Erythromycin, Ceftizoxime, Ciprofloxacin >Amoxicillin > Piperacillin + Tazobactam > Meropenem > Sulbactum + Cefoperazone > Doxycycline > Vancomycin.

Table 13. Antibiotics Sensitivity against *Staph. Aureus* strains in percentage

Sensitivity Pattern	AMOXIL, CLAVULANICACID	AMOXICILLIN	CEPHRADINE	CEFACLOR	CEFTIZOXIME	CIPROFLOXACIN	ENOXACIN	ERYTHROMYCIN	MEROPENEM	GENTAMYCIN	DOXICYCILIN	VANCOMYCIN	SULPHAMETHAXAZOLE, TRIMRTHOPRIM	SULBACTUM, CEFOPERAZONE	PIPRACILLIN, TAZOBACTUM
Sensitive	47.72	47.72	47.72	42.85	42.85	26.84	27.27	24.25	31.81	31.81	22.23	**82.82**	28.59	28.59	28.59
Intermediate	0	0	0	6.65	6.65	12.55	59.09	11.11	20.45	4.54	17.17	8.68	19.9	19.9	19.9
Resistant	52.27	52.27	52.27	50.5	50.5	60.6	13.63	64.64	47.72	**63.63**	60.6	8.5	51.51	51.51	51.51

- **Staph.epidermidis**

The activity of Vancomycin was maximal against *Staph.epidermidis*. All other antibiotics have activity in decreasing order of Doxycycline > Amoxicillin, Amoxil + Clavulanic acid, Sulbactum + Cefoperazone, Piperacillin + Tazobactam > Sulphamethizole + Trimethoprim > Erythromycin, Meropenem, Cephradine > Ciprofloxacin, Ceftizoxime, Gentamycin. The resistance of *Staph.epidermidis* against both Erythromycin and Gentamycin is maximal. The other drug resistance was in the decreasing order of Sulphamethizole + Trimethoprim, Cilipenem, Ceftizoxime, Ciprofloxacin > Cephradine, Amoxicillin, Amoxil + Clavulanic acid >Sulbactum + Cefoperazone >Piperacillin + Tazobactam > Doxycycline > Vancomycin shown in Table 14, Figure 15.

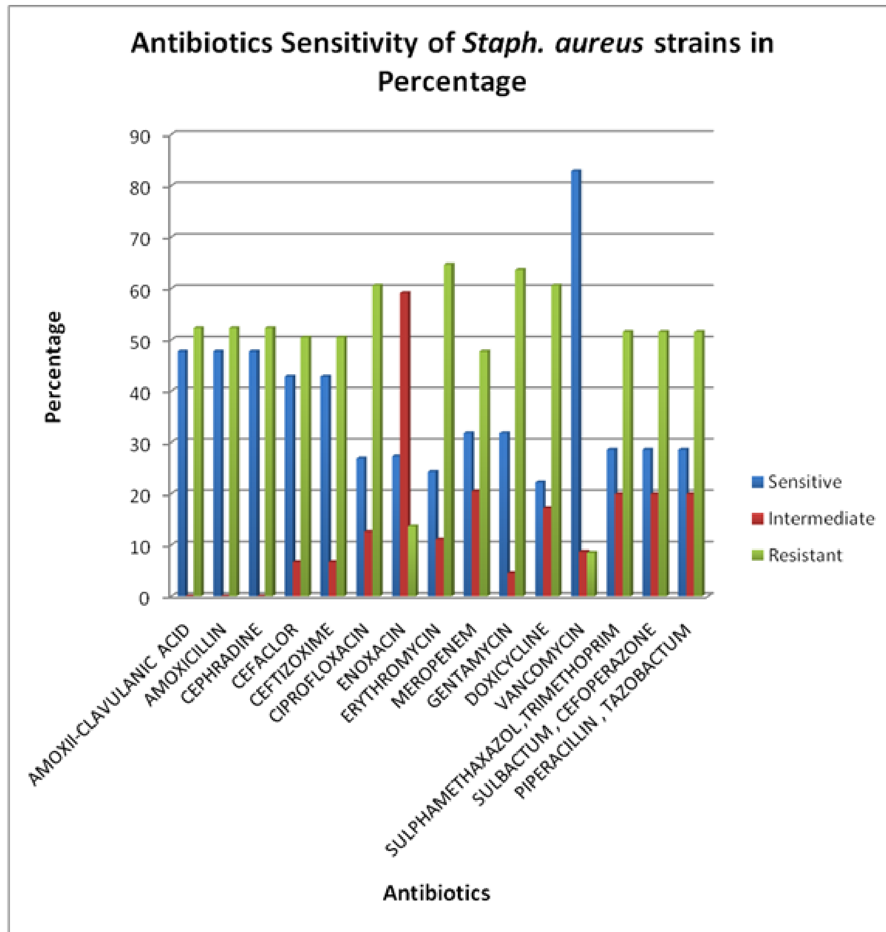

Figure 14. Antibiotics Sensitivity of *Staph. aureus* strains in percentage

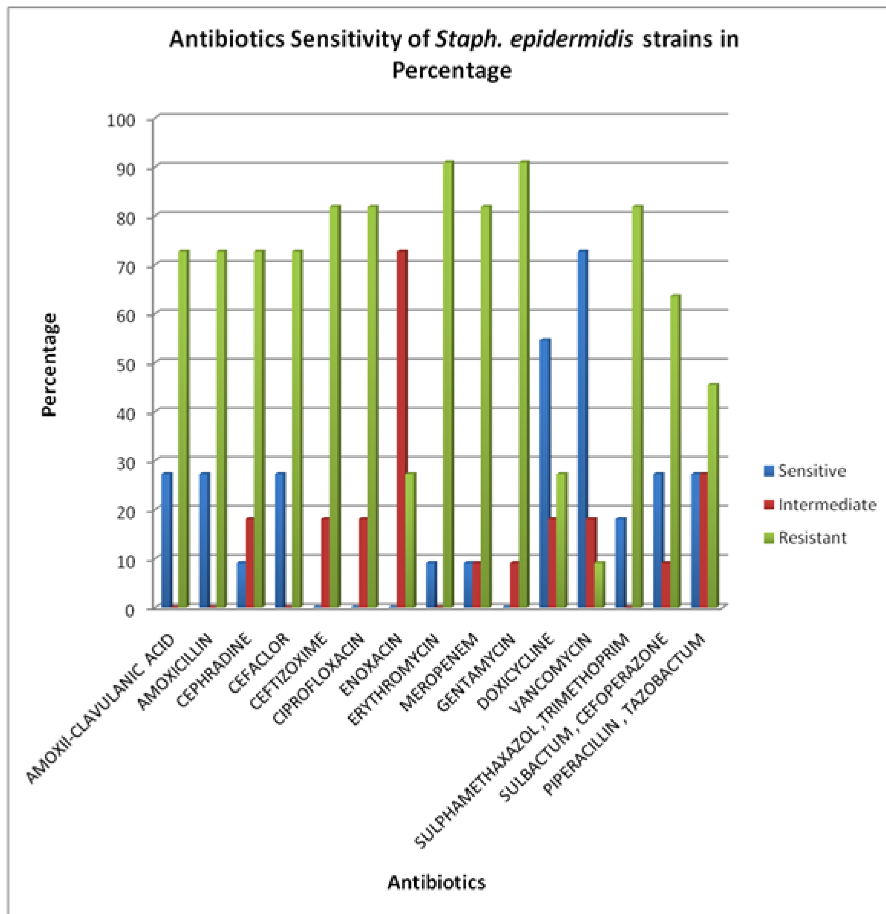

Figure 15. Antibiotics Sensitivity against *Staph. Epidermidis* strains in percentage

Table 14. Antibiotics Sensitivity against *Staph. epidermidis* strains in percentage

Sensitivity Pattern	AMOXIL, CLAVULANICACID	AMOXICILLIN	CEPHRADINE	CEFACLOR	CEFTIZOXIME	CIPROFLOXACIN	ENOXACIN	ERYTHROMYCIN	MEROPENEM	GENTAMYCIN	DOXICYCILIN	VANCOMYCIN	SULPHAMETHAXAZOLE, TRIMRTHOPRIM	SULBACTUM, CEFOPERAZONE	PIPRACILLIN, TAZOBACTUM
Sensitive	27.27	27.27	9.09	27.27	0	0	0	9.09	9.09	0	54.5	**72.72**	18.2	27.27	27.3
Intermediate	0	0	18.18	0	18.18	18.18	72.72	0	9.09	9.09	18.2	18.18	0	9.09	27.3
Resistant	72.72	72.72	72.72	72.72	81.81	81.81	27.27	**90.9**	81.81	**90.9**	27.3	9.09	81.8	63.63	45.5

5. Discussion

The existing study was aimed to assess the bacteriological number and types of computer accessories used in Hazara University and different diagnostic laboratories of Mansehra. 150 samples were collected by sterilizing swabs from different PCs used in different departments and different diagnostic laboratories of Mansehra. These samples were arranged in 3 different groups named as group "A", "B" and "C". For each sample serial dilutions were prepared for up to seven dilutions and cultivated on nutrient agar for evaluating the total number of bacterial counts. Similarly, samples were isolated and further confirmed by performing different biochemical tests such as Indole test, Coagulase test, Catalase test and MRVP test.

Out of 150 samples 70 samples were *E. coli* positive, 30 were *Klebsiella* positive, 25 were *S. aureus* positive and 25 were *S.epidermidis* positive. The reasons for these results were unhygienic conditions and multiple users. Even samples collected from single user computer keyboards and mouse also gave uncountable microbes. Most enteric pathogenic bacteria, including *E. coli*, *Klebsiella*, *S. Areas* have a high prevalence as a contaminant because they shed from the body, clothing, beddings and nostrils to the nearby areas and easily discharge by several human activities including talking, sneezing and physical contact with moist skin (Itah and Ben, 2004). That's why associated with numerous diseases like urinary tract, gastrointestinal (diarrhea) and nosocomial infections. Along with these, organisms carried by the wind can be moved from users to the surroundings (Oluduro *et al.,* 2011). While *S.epidemidis* infrequently presupposes an opportunistic pathogen, has a role in causing human infections like endocarditis (Anastasiades *et al.,* 2009). So, places like offices, laboratories, internet café, customer service departments, etc. surrounded by a lot of people moving in and out, are likely to have a large number of people sick due to skin dermatitis, seasonal infections, allergies, sneezing and hypersensitivities (Tagoe and Kumi-Ansah, 2011).

Antibiotic sensitivity was also performed to check their activity against specific antibiotics. The activity of Meropenem against *E.coli* was maximum 67.22% and showed maximum resistance about 97.36% against Erythromycin shown in Table 11, quite similar to the findings of early workers. They reported 77% resistance of Amoxil + Clavulanic acid, 76% Sulphamethaxazole + Trimethoprim and 68% for Ciprofloxacin while our readings were 81%, 77.5% and 66% respectively (Ejaz *et al.,* 2011).

The activity of Meropenem was maximal about 72.72% against *Klebsiella* and resistance was maximal in Amoxil + Clavolunic acid about 83.83% as shown in Table 12, very near to the earlier findings i.e. 81.81% resistance (Kacmaz and Sultan, 2007). *Staph aureus* showed maximal activity against Vancomycin about 82.82% and maximal resistance against Erythromycin about 64.64% as shown in Table 13 while Mehdinejad *et al.,* in 2008 had been reported that *Staph. aureus* has maximal activity against Vancomycin about 92.5% and maximal resistance against Erythromycin about 62.4%, very close to our results. Vancomycin had shown maximal activity of 72.72% against *Staph. epidermidis* and its resistance was 90.9% against both Erythromycin and Gentamycin (Table 14).

The resistance against antibiotic have many reasons such as incomplete or in sequence course of treatment, multiple use of antibiotics both for G +ve and G –ve bacteria, OTC drugs like antibiotics without any prescription, unethical approach of marketing, low trading potency (less than 100% results) and the most important cause is self medication (Tenover, 2006). So, it is recommended that proper diagnosis, clinical tests, starts from lower potency to higher potency and natural food therapy is required.

6. Conclusion

In conclusion, the isolation of the bacteria from computer accessories is a clear indication that the methods of sterilization or aseptic procedures adopted by the operators are less/not effective in significantly reducing the level of the pathogenic microbes on these surfaces to an acceptable level. It is also noticed that the level of knowledge among users in computer centers about the presence of microbes on computer accessories and their cleanliness is very poor.

So, it is strongly recommended that proper disinfectant like alcohol should be used on a regular basis for cleaning of computers and their surroundings to reduce the microbial load, especially for multiple-user sites. Hand hygiene before and after use of computers must be done by the students and public awareness programs should be encouraged (Rutala *et al.*, 2006; Enemuor *et al.*, 2012).

Acknowledgment

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Conflict of Interest

There is no conflict of interest between Authors or any organization that sponsored the research.

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Appendix 1. Antibiotic Disc Zone Diameter Interpretive Chart

Antibacterial Agent	Disc Potency (mcg)	Resistant (R)	Intermediate (I)	Sensitive (S)
Amoxi-Clave(Amoxicillin + Clavulanic Acid)	30	<19	-	>20
Effimox (Amoxicillin)	25	<11	-	>12
caydine (Cephradine)	30	<14	15-17	>18
Cefizox (Cefotaxime Sodium)	30	<10	11-19	>20
Ciprozan (Ciprofloxacin)	5	<15	16-20	>21
Ecin (Erythromycin)	15	<13	14-17	>18
Cilipenem (Meropenem)	10	<19	20-26	>27
Genta (Gentamycin)	10	<12	13-14	>15
Doxil (Doxycycline)	30	<12	13-15	>16
Vancorin (Vancomycin)	30	<12	13-15	>16
Actum (Sulbactum + Cefoperazone)	105	<15	16-20	>21
Cotrim (Trimethoprim + Sulphamethoxazole)	25	<10	11-15	>16

Appendix 2. Antimicrobial Discs and their potencies used in this study

S. #	Generic Name	Brand Name	Antibiotic Group	Code	Disc Potency (mcg)
1.	Erythromycin	Ecin	Macrolides	**E**	**15**
2.	Meropenem	Cilipenem	Carbapenem	**MEM**	**10**
3.	Gentamycin	Genta	Aminoglycosides	**CN**	**10**
4.	Doxycycline	Doxil	Tetracycline	**DO**	**30**
5.	Vancomycin	Vancorin	Glycopeptides	**VA**	**30**
6.	Chloramphenicol	Chloroptic	Protein synthesis inhibitor	**C**	**30**
7.	Amoxicillin + Clavulanic Acid	Amoxi-clave	Penicillin	**AMC**	**30**
8.	Amoxicillin	Effimox	Penicillin	**AML**	**25**
9.	Cephradine	Caydine	Cephalosporins	**CE**	**30**
10.	Ceftizoxime Sodium	Cefizox	Cephalosporins	**ZOX**	**30**
11.	Ciprofloxacin	Ciprozan	Quinolones	**CIP**	**5**
12.	Ampicillian	Amicil	Penicillin	**AM**	**10**
13.	Imipenem	Tienam	Cell Wall Synthesis Inhibitor	**IPM**	**10**
14.	Nalidixic Acid	Negram	Quinolones	**NA**	**30**
15.	Ofloxacin	Foraxin	Quinolones	**OFX**	**5**
16.	Trimethoprim + Sulphamethoxazole	Cotrim	Folic Acid Inhibitor	**SXT**	**25**
18.	Cefoperazone + Sulbactam	Actum	Cephalosporins	**SCF**	**105**
19.	Piperacillin + Tazobactam	Tazobact	Penicillin	**TZP**	**110**