Assessment of Gram-negative Bacteria on Computer Mouse at Sudanese Universities, Khartoum State

A dissertation submitted in partial fulfillment for the requirements of MSc in Medical Laboratory Science (Microbiology)

By:

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قال تعالى:

(وَمَا تَوَفِّيَّ فِي إِلَّا بِاللهِ عَلَيْهِ تَوَكَّلْتُ وَإِلَيْهِ أُنِيبُ)

صدق الله العظيم

سورة هود: الآية 88
DEDICATION

To my parents, supervisor and friends
ACKNOWLEDGEMENTS

First of all thanks to ALLAH for giving me the power and willing to complete this study.

Great thanks to my supervisor Prof. Humodi A. Saeed for his supervision and unlimited support during this work.

Thanks a lot to all universities authorities that involved in this study for their wonderful co-operation.

Thanks are extended to my teachers and laboratory technicians, especially Miss. Sohair Ramadan of the College of Medical Laboratory Science for their technical assistance.

Finally, all love and thanks to my friends for their fruitful comments and discussion.
Computers are ubiquitous and have been shown to be contaminated with potentially pathogenic bacteria in some communities. This study was undertaken to assess Gram-negative bacteria on computer mouse at Sudanese universities in Khartoum State. The study was conducted during the period from April to May, 2014.

The computer mice were aseptically sampled using moistened sterile cotton swabs. The swabs were immersed in 2 ml sterile normal saline, and then 1 ml was taken for counting bacterial load using pour plate technique. Identification of Gram-negative bacteria was done by colonial morphology and fermentation pattern on MacConkey's agar, Gram’s stain and conventional biochemical tests.

Out of 200 computer mice investigated, 123 (61.5%) yielded bacterial growth and 77 (38.5%) failed to demonstrate any bacterial growth. The average of bacterial load on computer mice was 56.26 \times 10^4 \text{ CFU/ml}. The results revealed that the CFU/ml were as follows: Neelin University 61.06 \times 10^4, Al_mogtarbeen University 60X10^4, University of Science and Technology 57X10^4, Sudan University of Science and Technology 54.78X10^4 and Khartoum University 43.6X10^4.

Fifteen (15)Gram-negative bacteria were identified. These were Enterobacter spp. 10 (66.7%), Pseudomonas spp. 2 (13.3%), Serratia spp. 2 (13.3%) and Klebsiella spp. 1 (6.7%).

The study concluded that the contamination rate of computer mice was high. Clinically important Gram-negative bacteria were recovered from computer mice.
Hand-washing should be adopted before and after using the computers to reduce the microbial contamination. Computer mice should be cleaned with alcohol or other disinfectants on a regular basis.
المستخلص

أجهزة الحاسوب موجودة في كل مكان، وقد أثبت أنها ملوثة بالأبكتيريا المسببة للأمراض محتملة في بعض المجتمعتين. وقد أجريت هذه الدراسة لتقييم الباكتيريالالسالبيةجرام على فأرة الحاسوب في الجامعات السودانية في ولاية الخرطوم. وقد أجريت الدراسة خلال الفترة من أبريل إلى مايو عام 2014.

وأخذت عينات من فأرة الحواسيب تحترشف معقم باستخدام قطعة قطن معقم مبلل. كانت مغروقة في مصبات 2 مل محلل ملحي معقم، ثم أخذ 1 مل حساب الباكتريات باستخدام طريقة صب الطب. وقد تم التعرف على البكتيريا السالبة جرام بواسطة شكل المستعمات البكتيرية والتخمير على أجار ماكوني. صبغة غرام والاختبارات الكيميائية الحيوية التقليدية.

من أصل 200 فأرة الحواسيب المفحوصة، 123 (61.5%) حققت نمو باكتريي و 77 (38.5%) فشل في إظهار أي نمو باكتريي. وبلغ متوسط الباكتريات لكل فأرة الحواسيب 57.6×10^4 وحدة مستعمرة باكتريية/مل. وكشفت النتائج أن وحدة مستعمرة باكتريية / مل كانت على النحو التالي: جامعة النيلين 61.06×10^4، جامعة المغتربين 60×10^4، جامعة العلوم والتقنية 54.78×10^4 و جامعة الخرطوم 43.6×10^4.

وقد تم تحديد خمسة عش باكتريات (15) السالبة جرام. كانت هذه الأمعانية 10 (66.7%)، الزائنة 2 (13.3%)، السرائية 2 (13.3%) والكليبية 1 (6.7%).

وخلصت الدراسة إلى أن معدل التلوث فيفأرة الحواسيب كان مرتفعاً. وتم عزل باكتيريا السالبة جرام من فأرة الحواسيب ذات أهمية سريرية.

ينبغي اعتماد غسل اليدين قبل وبعد استخدام أجهزة الحاسوب للحد من التلوث الميكروبي. يجب تنظيف فأرة الحاسوب بواسطة الكحول أو المطهرات الأخرى على أساس منتظم.
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CHAPTER ONE

INTRODUCTION AND OBJECTIVES
CHAPTER ONE

1. INTRODUCTION AND OBJECTIVES

1.1. Introduction

Computer is an electronic data processing machine which accepts data from the outside world in the form of an input and manipulates, calculates, computes on the basis of set of instructions supplied and stored in the memory and gives the required or desired results in the form of an output to the user (Ravichandran, 2006).

Because of frequent-dermal contact by numerous users, microbial reservoirs of interest include the computer keyboard and mouse (Neely et al., 2005; Wilson et al., 2006).

Furthermore, microorganisms found to contaminate fomites have also been shown to persist on environmental surfaces for varying periods of time ranging from hours to months and it has also been illustrated that they can still be detected and recovered from surfaces after routine conventional cleaning (French et al., 2004). In addition, cross infection of microorganisms between environmental surfaces and a host has equally been established (Hardy et al., 2006).

Some authors have demonstrated such contamination on the computer keyboard and mouse (Steffen et al., 2008). Concern has been raised that contact with contaminated computer keyboards might serve as a mechanism for contaminating the hands with potential pathogens, leading to cross-contamination of users (Steffen et al., 2008; Anderson & Palombo, 2009).

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 (Al-Ghamdi et al., 2011).

The presence of viable pathogenic bacteria on inanimate objects has been reported by earlier investigators. Several studies of the human environment have demonstrated colonization and contamination of objects such as door handles, faucets, phones, money, fabrics and plastics (Oluduro et al., 2011). Computers continue to have an increased presence in almost every aspect of our occupational, recreational, and residential environments. In the university environment, students have indicated that 100% have access to computers, 92.1% regularly use the Internet, and 73.3% regularly use e-mail. To accommodate the extensive use of computer technology, Universities have developed multiple-user “computer laboratories” on campus for general student access (Anderson & Palombo, 2009).

The increased availability of multiple-user computers in the organization 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 mouse is not yet fully understood. No clear legislation or even widely recognized guidelines have been formulated on the hazard caused by computer components (Kumar & Srivastava,
this is not in the best interest of campus students especially that computer keyboards and mice could spread significant number of pathogens (Enemuor et al., 2012).

1.2. Rationale

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 (Al-Ghamdi et al., 2011).

Owing to this indispensable nature of computer to the various activities of man in this technologically dominated society, there is increasing rate of interactions with the computer from day to day (Awe et al., 2013; Anderson and Palombo, 2009).

It is essential to identify the extent to which the people who continually interact with computer mouse are aware of the risk associated with its possibilities as a portal of infection (Awe et al., 2013). So the present study aimed to investigate the presence of Gram-negative bacteria on computer mouse that are frequently used by students in the Sudanese Universities, Khartoum.

1.3. Objectives

1.3.1. General objective

To assess Gram-negative bacteria on computer mouse at Sudanese universities in Khartoum State.
1.3.2. Specific objectives

1. To determine bacterial load on computer mouse.

2. To isolate Gram-negative bacteria from computer mouse.

3. To identify Gram-negative bacteria isolated from computer mouse.

4. To determine percentages of Gram-negative bacteria isolated from computer mouse.
CHAPTER TWO
LITERATURE REVIEW
CHAPTER TWO

2. LITERATURE REVIEW

2.1. Computer

Computer has been described as the latest technological media which are capable of receiving and accepting data, and performing operation according to instruction (program), and providing result of the operation with great speed and accuracy. The importance of computer had been identified in various fields such as Health, Agriculture, Finance, Education and Research institution (Awe et al., 2013).

Computers continue to have an increased presence in almost every aspect of our occupational, recreational, and residential environments (Anderson and Palombo, 2009).

In recent times, computer keyboards and mice are environmental objects in constant use with the growing need for computer system applications. Computer Keyboards and mice are components of a computer system that are used on daily basis in accomplishing various computer tasks in almost every aspect of our society. Their uses have greatly expanded and can be found in schools, banks, cybercafés, offices and hospitals (Joga and Palombo, 2012).

Computer hardware has been implicated as a potential reservoir for infectious agents. Computer terminals are well-recognized potential vehicles for the transmission of pathogenic bacteria. Contamination of keyboards and mice with bacteria, including staphylococci, enterococci, and coliforms, has been documented in health care and non-health care settings (Joga and Palombo, 2012).
2.2. **Bacterial contaminations**

Microorganisms are everywhere, bacteria and fungi contaminate our body, our houses, workplaces, pets and the whole environment. Fortunately, among many billions of bacteria, only 1,500 can be dangerous for our health, causing different diseases such as pneumonia or skin infections. The real problem is that the number of bacterial strains which develop resistances towards disinfectants and especially antibiotics is increasing very fast. Some of these resistant microorganisms are difficult to destroy and can survive for a longer time on the floor and other surfaces. European Congress Clinical Microbiology and Infectious Diseases reported that there are no safe objects (Eltablawy and Elhifnawi, 2009).

In various university environments, students have indicated 100% access to computers, 92.1% regularly use internet and 73.3% regularly use e-mail. To accommodate the intensive use of computer technology, universities have developed multiple-user “Cyber Cafe” on campus for general student access. 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 (Enemuor *et al.*, 2012).

Bacterial presence has been revealed also on mobile phones and even on the mouse and keyboard of personal computers. All these items and surfaces can be potential source for cross infections, transmitting microorganisms. Therefore, we should promote regular decontamination of daily use objects as part of an effective strategy to prevent the spread of multiresistant pathogens and the occurrence of consequent infections (Eltab lawy and Elhifnawi, 2009).
The main cause of bacterial contamination of computer keyboards and mice is eating lunch while working so crumbs and spills can wind up on and between the keys; the food deposits encourage the growth of millions of bacteria. Another cause is thought to be poor personal hygiene such as neglecting to wash hands after going to the bathroom. Dust, also which can trap moisture and enable any bacteria that are already on your keyboard to flourish. One potential cause of a keyboard that can make a person sick, is sharing it among other workers. One of whom may have coughed or sneezed into his hand (Eltablawy & Elhifnawi, 2009).

The ubiquitous sharing of public computers by a broad user base might facilitate increased transmission and prevalence of pathogenic microorganisms throughout the community. Inadequately performed hand hygiene and non-disinfected surfaces are two reasons why the keys and mouse-buttons of laptops could be sources of microbial contamination resulting consequently in indirect transmission of potential pathogens and nosocomial infections (Anyim et al., 2013).

Surprisingly, little effort has been dedicated to identify the role of inanimate surfaces as pathogen reservoirs in the non-hospital settings. Therefore, successive steps to edge the spread of antimicrobial resistant pathogens throughout the community should include efforts to not only increase awareness of appropriate hygiene and decontamination strategies, but also to reveal the ecology of bacteria contaminating community surfaces (Anyim et al., 2013).

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 (Anyim et
Infectious doses of pathogens may be transferred to the mouth after handling an everyday contaminated household object (Rusin et al., 2002).

Bacterial contamination of keyboards and mice pose as a threat to public health as bacteria can be transferred from person to person, by direct contact or indirect contact via an inanimate object and back again. Computer keyboard and mouse are capable of hosting pathogenic microbes, and hence been able to act as a portal of infection (Awe et al., 2013).

People are exposed to this risk unconsciously because of the low level of awareness among users of computers, this thus serve as a medium to inform users of keyboard and mouse about the necessary need to be more careful as they interact with this wonderful instrument of technology (Awe et al., 2013).

It was found that all the tested computer keyboards and mice were positive for microbial contamination. Most of these isolates were traditional skin flora. In addition other organisms such as Gram-positive rods, cocci and mould, revealed a general level of contamination of these widely used equipments (Eltablawy and Elhifnawi, 2009).

In the study of Eltablawy and Elhifnawi, (2009) the contamination rate of keyboards and mice was 99.9% and 100%, respectively. The sampled potentially pathogenic microorganisms contained 3.0% of *Bacillus cereus*, *Pseudomonas putida* and *Escherichia tarda* on computer keyboards when compared with that on mice (0.0%).
While in the study of Anyim et al. (2013) the percentages of *Escherichia spp.* and *Pseudomonas spp.* were 34.1% and 18.2%, respectively.
CHAPTER THREE
MATERIALS AND METHODS
CHAPTER THREE
3. MATERIALS AND METHODS

3.1. Study design

3.1.1. Type of study

This study is a cross-sectional study (descriptive study), was conducted in the computer laboratories in Sudanese universities in Khartoum State during the period from April to May, 2014.

3.1.2. Study area

This study was conducted in the computer laboratories in Sudan University of Science and Technology, Neelain University, Al_Moghtaribeen University of Science and Technology, University of Khartoum and University of Science and Technology.

3.1.3. Study duration

This study was conducted during the period from April to May, 2014.

3.2. Ethical consideration

The approval has been taken from all computer laboratories authorities among the Sudanese Universities in Khartoum State and we were agreed to the confidentiality of the results.
3.3. **Laboratory procedures**

The laboratory procedures such as sample collection, sample processing, pour plate technique, culture, macroscopically and microscopically examinations and biochemical tests were used to determine colony count, isolation and identification of Gram-negative bacteria.

3.3.1. **Collection of samples**

The specimens were collected by using sterile cotton swab, firstly moistened in sterile normal saline, then the computer mouse was swabbed gently by rolling the swab over the entire surface of the mouse, particularly the right and left click parts. After that each swab was placed in the 2 milliliter of sterile normal saline on the sterile plain container, mixed well and the wooden stick of the swab was broken to fit the closing of container carefully.

3.3.2. **Viable count**

Ten-fold serial dilution of the specimen was made as following:

Nine milliliter of sterile normal saline (appendix 7) were placed in four sterile glass test tubes $10^1$, $10^2$, $10^3$ and $10^4$, and then from the well mixed sample by using vortex 1 ml was taken and added to the 9 ml in the first tube, after that the serial dilution was done.

The autoclaved melted nutrient agar media was permitted to cool at about 45°C. From melted nutrient agar 15 ml were mixed with 1 ml of well mixed serial dilution from the second ($10^2$), third ($10^3$) and fourth ($10^4$) tubes on the sterile plastic disposable
petridishes. Two sterile plastic disposable petridishes were used for each dilution. Then the petridishes were mixed thoroughly by tilting and swirling. After that the plates were allowed to solidify at room temperature (Protocol no. 1).

After incubation period at 37°C for overnight, the colonies were counted on the dilution that gives count between 30 to 300 colonies.

Calculation

The counted colonies were calculated according to the David's protocol:

\[ \text{CFU} \times \text{dilution factor} \times \frac{1}{\text{aliquot}} = \text{CFU/ml} \].

3.3.3. Bacteriological methods

3.3.3.1. Inoculation

Under aseptic conditions (near Bunsen burner) from Nutrient agar 3-5 identical colonies were sub-culured onto MacConkey agar (appendix 1) by using sterile wire loop, firstly well area was done and streaked on three areas followed by zigzag.

3.3.3.2. Incubation

The inoculated plates were transferred to incubator and incubated at 37°C for 24 hours.

3.3.3.3. Isolation

The growth on MacConkey agar exhibited significant growth was cultured on the slope of Nutrient agar (appendix2) by using sterile straight wire in zigzag form for further identification.
3.3.3.4. Identification

The isolates were identified by the following techniques:

3.3.3.4.1. Colonial morphology

The comment on colonial morphology on MacConkey's agar growth was performed with regard to the size, appearance, color, edge, side view, smell and pigment production of the colonies.

3.3.3.4.2. Gram's stain

The Gram's staining reaction (appendix 8) is used to help identify pathogens in specimens and cultures by their Gram's reaction and morphology (Cheesbrough, 2000).

Gram-negative bacteria stain red because after being stained with crystal violet they are decolorized by ethanol (Cheesbrough, 2000). And the procedure is as following: The dried smears were prepared by placing small drop of sterile normal saline onto glass slide, and then by using sterile wire loop the colony was touched and added to the drop, mixed and spreaded in circular manner. The fried smears were fixed by pass the slide 3 times through flame. The fixed smear was covered with crystal violet stain for 30-60 seconds. The stain was rapidly washed off with clean water. All the water was tipped off, and the smear was covered with Lugol's iodine for 30-60 seconds. The iodine was washed off with clean water. The smear was rapidly decolorized with acetone-alcohol for few seconds. And then immediately washed with clean water. The smear was covered with safranine stain for 2 minutes. After that the stain was washed
off with clean water. The back of the slide was wiped with clean gauze and placed in a draining rack for the smear to air-dry. Finally; the stained smears were examined microscopically, first with the 40x objective to check the staining and to see the distribution of material, and then with the oil immersion objective to report the bacteria.

3.3.3.4.3. Biochemical tests

3.3.3.4.3.1. Indole test

Testing for indole production is important in the identification of enterobacteria. And this test based on break down of the amino acid tryptophan with the release of indole. Indole production is detected by Kovac's reagent (appendix 9) to produce a red coloured ring.

Under aseptic condition, by using sterile wire loop, the peptone water (appendix 3) was inoculated with the testing organism and incubation was done at 37°C for overnight (Cheesbrough, 2000).

3.3.3.4.3.2. Urease test

Testing for urease enzyme activity is important in differentiating enterobacteria. When the strain is urease producing, the enzyme will break down the urea in the urea agar to give ammonia and carbon dioxide. With the release of ammonia the medium becomes alkaline as shown by a change in colour of the indictor to pink-red.
Under aseptic condition, by using sterile straight wire, the Urea agar (appendix 4) was inoculated with testing organism and incubation was done at 37˚c for overnight (Cheesbrough, 2000).

### 3.3.3.4.3.3. Citrate utilization test

This test used occasionally to assist in the identification of enterobacteria. The test is based on the ability of an organism to use citrate as its only source of carbon.

Under aseptic condition, by using sterile straight wire, slope of Simmon's citrate (appendix 5) was inoculated with organism under test, and incubation was done at 37˚c for overnight (Cheesbrough, 2000).

### 3.3.3.4.3.4. Fermentation of sugar and production of H₂s and gas

This test is used for differentiating the enterobacteria. And the test is based on the glucose and lactose fermentation, gas and hydrogen disulfide production.

Under aseptic condition, by using sterile straight wire, the KIA (appendix 6) was inoculated with testing organism, first the butt was stabbed then the slope was streaked, and incubation was done at 37˚c for overnight (Cheesbrough, 2000).

<table>
<thead>
<tr>
<th><strong>Bacteria</strong></th>
<th><strong>Slope</strong></th>
<th><strong>Butt</strong></th>
<th><strong>Gas</strong></th>
<th><strong>H₂S</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Klebsiella spp.</td>
<td>Yellow</td>
<td>Yellow</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Enterobacter spp.</td>
<td>Yellow</td>
<td>Yellow</td>
<td>+</td>
<td>_</td>
</tr>
<tr>
<td>Serratia spp.</td>
<td>Red or yellow</td>
<td>yellow</td>
<td>different</td>
<td>-</td>
</tr>
<tr>
<td>Pseudomonas spp.</td>
<td>Red</td>
<td>Red</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
3.3.4.3.5. Preservation of isolated organisms

All isolated organisms were preserved in sterile nutrient agar slopes at 4-8°C.

3.4. Quality control

All precautions were considered carefully; including working under aseptic conditions and the sterility of all materials were used.

The sterility of normal saline was tested by inoculating two drops of normal saline onto sterile nutrient agar plate and incubated at 37°C for 24 hours. Whereas the sterility of nutrient agar was checked by incubating one plate for each batch (20 plates) of nutrient agar at 37°C for 24 hours.
CHAPTER FOUR
RESULTS
CHAPTER FOUR

4. RESULT

A total of two hundreds (200) computer mice swabs were collected from different Sudanese Universities in Khartoum State. Of these 49 samples from Sudan University of Science and Technology, 42 from Alneelin University, 37 from Khartoum University, 41 from University of Science and Technology and 31 from Almogtarbeen University (Table 1).

The swabs were cultured on nutrient agar. Of these 123 (61.5%) samples were exhibited bacterial growth, whereas 77 (38.5%) samples were demonstrated no bacterial growth (Fig.1). The load of contamination was expressed in the term of colony-forming unit per ml (CFU/ml).

The results revealed that the CFU/ml were as follows: Sudan University of Science and Technology 54.78X10^4, Neelin University 61.06X10^4, Khartoum University 43.6X10^4, University of Science and Technology 57X10^4 and Almogtarbeen University 60X10^4. The average of bacterial load on computer mice among Sudanese universities in Khartoum State was 56.26 X 10^4 CFU/ml. (Table 1).

By using Gram's stain for primary identification, 15 (12.2%) isolates were found Gram-negative bacilli, 55 (44.7%) Gram-positive cocci and 53 (43.1%) Gram-positive bacilli (Fig.2).

A total of 15 Gram-negative bacilli were cultured on MacConkey's agar. Of these 13 Gram-negative bacilli were lactose fementors, whereas 2 Gram-negative bacilli were non-lactose fermentors.
A set of biochemical tests were adopted to confirm identification of isolated Gram-negative bacilli (Table 2). The identified Gram-negative bacteria were as follows: \textit{Enterobacter} spp. 10 (66.7\%), \textit{Pseudomonas} spp. 2 (13.3\%), \textit{Serratia} spp. 2 (13.3\%) and \textit{Klebsiella} spp. 1 (6.7\%) (Table 3).
Fig. 1. Percentage of bacterial growth on Nutrient agar medium after overnight incubation.

Fig. 2. Result of Gram's reaction of isolated bacteria from computer mice.
Table 1. Bacterial load on computer mice assessed in Sudanese universities in Khartoum State

<table>
<thead>
<tr>
<th>University</th>
<th>Total No</th>
<th>Growth</th>
<th>Mean of count (CFU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alneelin University</td>
<td>42</td>
<td>29</td>
<td>61.06 X 10^4</td>
</tr>
<tr>
<td>Almogtarbeen University</td>
<td>31</td>
<td>21</td>
<td>60 X 10^4</td>
</tr>
<tr>
<td>University of Science and Technology</td>
<td>41</td>
<td>25</td>
<td>57 X 10^4</td>
</tr>
<tr>
<td>Sudan University of Science and Technology</td>
<td>49</td>
<td>33</td>
<td>54.78 X 10^4</td>
</tr>
<tr>
<td>Khartoum University</td>
<td>37</td>
<td>15</td>
<td>43.6 X 10^4</td>
</tr>
</tbody>
</table>

Table 2. Biochemical tests for the isolated Gram-negative organisms

<table>
<thead>
<tr>
<th>Organism</th>
<th>KIA</th>
<th>Indole test</th>
<th>Urease test</th>
<th>Citrate test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Butt</td>
<td>Slope</td>
<td>Gas</td>
<td>H₂S</td>
</tr>
<tr>
<td>Klebsiella spp.</td>
<td>Y</td>
<td>Y</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Serratia spp.</td>
<td>Y</td>
<td>Y</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Enterobacter spp.</td>
<td>Y</td>
<td>Y</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Pseudomonas spp.</td>
<td>R</td>
<td>R</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
Table 3. Frequency and percentages of isolated Gram-negative bacilli

<table>
<thead>
<tr>
<th>Organism</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Enterobacter</em> spp.</td>
<td>10</td>
<td>66.7%</td>
</tr>
<tr>
<td><em>Serratia</em> spp.</td>
<td>2</td>
<td>13.3%</td>
</tr>
<tr>
<td><em>Pseudomonas</em> spp.</td>
<td>2</td>
<td>13.3%</td>
</tr>
<tr>
<td><em>Klebsiella</em> spp.</td>
<td>1</td>
<td>6.7%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>15</strong></td>
<td><strong>100%</strong></td>
</tr>
</tbody>
</table>
CHAPTER FIVE
DISCUSSION
CHAPTER FIVE

5. DISCUSSION

5.1. Discussion

Numerous studies have indicated that computer keyboards (and mice) can become contaminated with pathogenic bacteria (Anderson & Palombo, 2009; Rutala et al., 2006).

In the present study, a total of 200 computer mice were examined for bacterial load and identification of Gram-negative bacteria. Of these 123 (61.5%) samples were exhibited bacterial growth, whereas 77 (38.5%) samples were demonstrated no bacterial growth. This result indicates that the contamination rate on computer mice was high. The result is less than that reported by Eltablawy and Elhifnawi (2009) who found that all samples taken from computer mice were contaminated (100%). But it is more than result reported by Anyim et al. (2013) who found that the contamination rate of computer mice was (29.7%). These differences may attribute to the levels of knowledge among computers users about hygiene practice is very poor.

The average of bacterial load on computer mice among Sudanese universities in Khartoum State was 56.26 X 10^4 CFU/ml. Chairman et al. (2011) reported that higher bacterial load from computer mice (2.91x10^9 CFU/ml). The bacterial load among universities in Khartoum State was represented in (Table 1). It was higher in Neelin University followed by Almogtarbeen University; 61.06X10^4 CFU/ml and 60X10^4 CFU/ml, respectively. This finding may attributed to the level of knowledge among the computer users in computer laboratories about the possibility of microorganisms on the computer mouse is very poor and high number of computer users. Such a high
level of contamination on user interfaces is worrisome because a relationship can be demonstrated between environmental contamination and the acquisition of bacteria by people (Hardy et al., 2006; Bures et al., 2001; Yuhuan et al., 2001).

Depending on microscopic, cultural examinations and biochemical tests 15 (12.2%) isolates of The Gram-negative bacteria were identified; 10 (66.7%) of Enterbacter spp., 2 (13.3%) isolates for both Serratia spp. and Pseudomonas spp., and 1 (6.7%) isolate for Klebsiella spp. (table 3). These results indicate that the computer mice might act as environmental vehicle for the transmission of potentially pathogenic bacteria in the university settings and also indicate the need for increasing awareness among computer users on cleaning of such surfaces or disinfection and adequate hand-washing hygiene (Ali et al., 2013).

The percentage of isolated Gram-negative bacilli in this study is in consistent with the finding of Al-Ghamdi et al. (2011) who found that (11%) Gram-negative bacilli isolated from computer mice.

The isolation of Pseudomonas spp. and bacteria of Enterobacteriaceae family are ubiquitous and that these organisms can be shed from the body, clothing, bedding, nostrils and carried in the dust to other surfaces (Itah & Ben, 2004), hence their presence on the computer mice. This result is totally convenient with the result of Ali et al. (2013) who isolated 2 of Pseudomonas species. The same result was shown by Tagoe and Kumi-Ansah (2011) is in consistent with this study, showing that Pseudomonas spp. can be isolated from computer mouse. Hence Infectious doses of this pathogen may be transferred to the mouth after handling an everyday contaminated object.
Enterbacter spp. was recorded that higher percentage among isolates in university settings in Khartoum State. This result disagrees with the result of Oluduro et al. (2011) who isolated (4.9%) Enterbacter spp. which represents the lowest isolated bacterium from environmental objects. Of particular interest was the isolation of Serratia spp., which is not reported by anyone in previous studies on computer mice. It appears that this is the first report of Serratia spp. to be isolated from computer mouse.

5.2. Conclusion

High contamination rate of computer mice was detected. The contaminants were pathogenic and non-pathogenic bacteria. The highest bacterial load was reported in Neelin University \((276 \times 10^4 \text{ CFU/ml})\) whereas the lowest bacterial load was reported in both Neelin and Khartoum universities \((2 \times 10^4 \text{ CFU/ml})\). All identified Gram-negative bacteria were potentially pathogenic bacteria. They are common cause of pneumonia and urinary tract infections when the immunity of those users is diminished. Therefore this device is potential vehicle for the transmission of clinically important pathogens. Enterbacter spp. was recorded that higher percentage among isolates in university settings in Khartoum State. Serratia spp. was being first reported from samples taken from computer mice in this study.
5.3. **Recommendations**

1. Routine cleaning of computer mice may aid the fight against pathogens in various communities.

2. Hand-washing before and after using the computers is highly recommended to reduce the microbial contamination of computer mice.

3. Using alcohol or suitable disinfectants which have major role in reducing the level of contamination.

4. Further studies are required to validate the results of present study by taking larger sample size from different universities according to the geographical distribution in Khartoum state.
REFERENCES
REFERENCES


APPENDICES
APPENDICES

All media are prepared from Himedia Laboratories pvt, Ltd, made in India.

Appendix 1

MacConkey agar

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Grams/ Liter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptone</td>
<td>3.00</td>
</tr>
<tr>
<td>Pancreatic digest of gelatin</td>
<td>17.00</td>
</tr>
<tr>
<td>Lactose monohydrate</td>
<td>10.00</td>
</tr>
<tr>
<td>Bile salts</td>
<td>1.500</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5.00</td>
</tr>
<tr>
<td>Neutral red</td>
<td>0.03</td>
</tr>
<tr>
<td>Agar</td>
<td>13.50</td>
</tr>
</tbody>
</table>

**Preparation**

By dissolving 49.53 grams of medium in 1000 ml distilled water. The medium was sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minutes, cooled and poured in petridishes.
## Appendix 2

### Nutrient agar

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Grams/Liter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptic digest of animal tissue</td>
<td>5.00</td>
</tr>
<tr>
<td>Beef extract</td>
<td>1.50</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>1.50</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5.00</td>
</tr>
<tr>
<td>Agar</td>
<td>15.00</td>
</tr>
</tbody>
</table>

**Final pH 7.4 ± 0.2 at 25 °C**

**Preparation**

By dissolving 28 g of powder in 1000 ml distilled water. The medium was sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minutes, cooled and poured in petridishes.

## Appendix 3

### Trypton water

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Grams/Liter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptic digest of animal tissue</td>
<td>10</td>
</tr>
</tbody>
</table>
Sodium chloride 5

Final PH (25°C) 7.2

**Preparation**

By dissolving 15 g of powder in 1 L D.W then sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minutes, cooled and poured in tube.

**Appendix 4**

**Urea agar base**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Grams/Liter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gelatine peptone</td>
<td>1.0</td>
</tr>
<tr>
<td>D (+)-Glucose</td>
<td>1.0</td>
</tr>
<tr>
<td>Potassium dihydrogen phosphate</td>
<td>2.0</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5.0</td>
</tr>
<tr>
<td>Phenol red</td>
<td>0.012</td>
</tr>
<tr>
<td>Agar</td>
<td>12.0</td>
</tr>
</tbody>
</table>

Final pH 6.8 +/- 0.2 (at 25°C)

**Preparation**

By dissolving 21 g in 950 ml distilled water, sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minutes. After cooling to 50°C, 50ml of sterile filtered 40% urea solution were added aseptically (Fluka 08582). Mixed well and let cooled as slants.
### Appendix 5

**Simmon's citrate**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Grams/Liter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium Citrate</td>
<td>2.00</td>
</tr>
<tr>
<td>Dipotassium Phosphate</td>
<td>1.00</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5.00</td>
</tr>
<tr>
<td>Magnesium Sulphate</td>
<td>0.20</td>
</tr>
<tr>
<td>Ammonium dihydrogen phosphate</td>
<td>1.00</td>
</tr>
<tr>
<td>Bromothymol blue</td>
<td>0.08</td>
</tr>
<tr>
<td>Agar</td>
<td>15.00</td>
</tr>
</tbody>
</table>

Final pH 6.8 ± 0.2 (at 25°C)

**Preparation**

By dissolving 24.28 grams of the medium in1000 ml distilled water, mixed well and dissolved by heating with frequent agitation, Boiled for one minute until complete dissolution and sterilized in autoclave at 15 lbs pressure (121°C) for 15 minutes, cooled and poured in tube as slopes.

### Appendix 6

**Kliglar's Iron Agar**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Grams/Liter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beef extract</td>
<td>3.00</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>3.00</td>
</tr>
</tbody>
</table>
Casein enzymic hydrolysate 20.00
Sodium chloride 5.00
Lactose 10.00
Glucose anhydrous 1.00
Ferrous ammonium sulphate, 6H2O 0.50
Sodium thiosulphate, pentahydrate 0.50
Phenol red 0.025
Agar 15.00
Final pH (at 25°C) 7.4±0.2

**Preparation**

By dissolving 57.52 grams of the medium in 1000 ml distilled water, mixed well and dissolved by heating with frequent agitation, Boiled for one minute until complete dissolution and sterilized in autoclave at 15 lbs pressure (121°C) for 15 minutes, allowed to cool and distributed into tubes. The tubes were allowed to cool in slanted position to form slopes with about 1 inch butts.

**Appendix 7**

**Physiological saline (0.85% w/v)**

**Ingredients**

Sodium chloride 8.5 g
Distilled water 1 L

**Preparation**
By dissolving 8.5 g of NaCl in 1000 ml distilled water in beaker. Then the beaker was covered with foil and Sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Appendix 8

Gram's stain

Ingredients

Crystal violet

Crystal violet 20g
Ammonium oxalate 9g
Ethanol absolute 95g
Distilled water 1L

Lugol's iodine

Iodine 10g
Potassium iodine 20g
Distilled water 1L

Safranin

Safranin 0.5g
Distilled water 100ml
Appendix 9

Kovac's reagent:

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Grams/Liter</th>
</tr>
</thead>
<tbody>
<tr>
<td>p-Dimethylaminobenzaldehyde</td>
<td>50 g</td>
</tr>
<tr>
<td>amylalcohol</td>
<td>750 ml</td>
</tr>
<tr>
<td>HCl 37%</td>
<td>250 ml</td>
</tr>
</tbody>
</table>
PURPOSE:
The pour plate technique can be used to determine the number of microbes/mL in a specimen.
It has the advantage of not requiring previously prepared plates, and is often used to
assay bacterial contamination of foodstuffs. The principle steps are to:
1) prepare and/or dilute the sample
2) place an aliquot of the prepared sample in a labeled empty sterile plate
3) pour 15 mL of melted agar, cooled to 45°C, into the plate, swirl to mix well
4) let cool to solidify (without disturbing)
5) invert and incubate to develop colonies (24-48 hours).
Each colony represents a "colony forming unit" (CFU). As usual, for accurate counts, the optimum
count should be within the range of 30 to 300 colonies/plate.
One disadvantage of pour plates is that embedded colonies are much smaller than those which
happen to be on the surface. Thus, one must be careful to score these so that none are
overlooked.
Also, obligate aerobes may grow poorly if deeply imbedded in the agar.

EQUIPMENT:
15 mL sterile Plate Count Agar (PCA) in capped 16 x 150 mm test tubes*
Hot Block, 45o C (or water bath)
3" deep to equal agar depth
sterile capped 16 x 150 mm test tubes
0.1, 1.0 and 2.0 mL pipets, sterile
petri dishes, empty and sterile
flame
colony counter with magnifying glass

POUR PLATE TECHNIQUE:
1. Construct a table in your notebook: details of preparing and plating your specimen(s): with a line for each plate which describes:
a: the detailed identity or source of the specimen
b: the dilution of the specimen used (prepare the dilution expected to contain between 30-
300 CFU/aliquot. Describe the method for its dilution/preparation.
c: the volume of diluted specimen (aliquot) you will plate (usually 0.1 to 1.0 mL)
2. Label the bottom of the plate with the above data plus initials, seat number and date.
3. Dilute specimen as written out in 1.b.
4. **Inoculate labeled empty petri dish** with specified mL diluted specimen (from 1. c.)

5. **Pour 15 mL of melted, 45° C Plate Count Agar** into the inoculated petri dish.

6. **Mix thoroughly by tilting and swirling the dish.** *Do not slop the agar over the edge of the petri dish.*

7. **Allow the agar to completely gel without disturbing it.** *(About 10 minutes).*

8. **Invert and incubate** at 37 °C for 24-48 hours.

9. **Count, record, calculate:** Count all colonies *(again: note that the embedded colonies will be much smaller than those which happen to form on the surface).* A magnifying colony counter can aid in counting small embedded colonies. Record the data. Calculate CFU/mL or CFU/g. Enter results in your table.

   \[
   [\text{CFU x dilution factor x 1/ aliquot} = \text{CFU/mL}]
   \]

* *For 600 mL of NA + 1% glu: 9 g agar, 4.8 g nutrient broth, 6 g dextrose. Dissolve ingredients at 95 °C, repipet into 16 x 150 mm tubes, cap, autoclave, 15 lb, 15 min. Cool to 45 °C before using. Plate Count Agar may also be used.*