ASSESSMENT OF MICROBIOLOGICAL CONTAMINATION ON COMPUTER KEYBOARDS IN OFFICE SETTINGS: A CASE STUDY AT MADONNA UNIVERSITY NIGERIA, ELELE, RIVERS STATE, NIGERIA

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Abstract:
Microorganisms inhabit various environments, displaying remarkable adaptability and proliferation capabilities. They colonize diverse surfaces, including those of computer keyboards, presenting potential health risks. This study investigates the prevalence of microbiological contamination on computer keyboards within office settings at Madonna University Nigeria, Elele, Rivers State, Nigeria. Swab samples were gathered and handled using standard microbiological techniques to distinguish between different types of bacteria and fungi. These microorganisms were then tested to see how they respond to commonly used antibiotics, and their genetic makeup was analyzed. The results showed the existence of harmful bacteria like *Staphylococcus aureus, Bacillus cereus, Enterobacter sp,* coagulase-negative *Staphylococcus sp, Salmonella sp.,* and *Escherichia coli*. Various fungal species such as *Penicillium sp., Aspergillus sp.,* and *Mucor sp*. were also acknowledged. Antibiotic susceptibility tests indicated diverse resistance patterns among bacterial isolates, including multidrug resistance. Molecular characterization identified *Bacillus cereus* among the isolates. These findings underscore the potential health hazards associated with contaminated computer keyboards, emphasizing the importance of consistent cleaning and disinfection protocols to mitigate Malady transmission in shared environments.

Keywords: **Assessment, Microbiological Contamination, Computer Keyboards, Office Settings, Madonna University, Nigeria**

1. Introduction

Microorganisms are incredibly adaptable and can proliferate rapidly in various environments, as highlighted by Oluduro et al. [1]. These can be observed across different surfaces and ecosystems, ranging from soil to acidic thermal springs, radiological wastewater, and even well beneath the lithosphere. Moreover, they reside in organic substances and the physical structures of plants and animals. While essential for ecological processes, some microorganisms can pose health risks, causing diseases like pneumonia and skin infections. A concerning issue is the rapid increase in bacterial strains resistant to disinfectants, especially antibiotics.

The European Society of Clinical Microbiology and Infectious Diseases (ECCMID) reported on the ubiquitous presence of microbial contamination on everyday objects, as cited in Anagboso et al. [2]. Items such as tables, utensils, computers, doorknobs, gym equipment, and even ATM keyboards are contaminated. This contamination poses a risk of cross-infections and transmission of microorganisms among users, especially in environments where interfaces are shared extensively.

The extensive integration of computer systems and interfaces across diverse environments such as educational institutions, medical facilities, internet cafes, and workplaces has facilitated frequent and unimpeded user sharing. This unrestricted sharing increases the likelihood of microorganisms originating from either human microflora or

the surroundings being transmitted between users who share these interfaces. Factors such as eating while working, poor personal hygiene, and dust accumulation contribute to microbial contamination on keyboards. Sharing keyboards among workers, particularly those who cough or sneeze into their hands, can transmit infections.

Even with computers becoming increasingly ubiquitous in daily life, there's a common misunderstanding that microbes are confined to research laboratories or healthcare environments. Yet, research has shown that everyday items such as computers, doorknobs, phones, and fabrics can become colonized and contaminated by microorganisms. In university environments, where computer usage is prevalent among students and staff, the risk of microbial transmission through shared interfaces is particularly high. Thus, awareness and measures to mitigate microbial contamination on interfaces are crucial in maintaining public health.

Previous research, as referenced in Huber and Pelon [3] and Buers et al. [4], has highlighted the capacity of computers to act as fomites in healthcare and hospital settings. Additionally, the contamination of office environments, encompassing computer peripherals like keyboards and mice, with microorganisms, has been acknowledged [5]. Infrequent disinfection of computers creates a significant opportunity for contaminating microorganisms to spread.

Although we are becoming more aware of how common microbes are in the environment, we still do not fully understand the exact level of risk that computer keyboards and mice provide.

Currently, no clear standards or widely accepted guidelines address the hazards associated with computer components, such as keyboards and mice. This lack of regulatory framework is concerning, particularly considering the potential for these components to harbor and disseminate many pathogens.

This gap in oversight is not in the best interest of computer users, as they could inadvertently spread pathogens through shared equipment. To address this issue, the study aimed to conduct an assessment of the antibiotic susceptibility profile of microorganisms present on computer keyboards in selected offices at Madonna University Nigeria, Elele, Rivers State, Nigeria. By examining the susceptibility of these microorganisms to antibiotics, the study sought to enhance our understanding of the health risks associated with contaminated computer keyboards and contribute to developing guidelines or regulations aimed at mitigating these risks for computer users.

2. MATERIALS AND METHOD

2.1 STUDY AREA

The investigation was executed on-site at Madonna University Nigeria in Elele, a town nestled within Rivers State, Nigeria. Elele Town resides in the southeastern region of Nigeria, characterized by its geographical coordinates of latitude 5° 27° - 5° and longitude 6° 55 -7° 85E. The climate in this area is typically tropical, marked by a mean daily temperature of approximately 29 °C throughout much of the year. Rainfall is a significant climatic feature, with the annual precipitation ranging between 217 and 240 centimeters.

Elele Town is bound by several other towns and villages, each contributing to the formation of the broader regional landscape. Among these adjacent areas are, Ogba/Ndoni/Egbema, Ahoada, Omagwa, Owerri Town, Omoku, and Isiokpo Town among others. The geographical context of the study gives information on the environmental conditions in which the research was carried out. This includes details about the climate, rainfall patterns, and regional topography, which can affect the existence and behaviour of microorganisms in the study area.

2.2 STERILIZATION OF MATERIALS

All glassware utilized in this research underwent a meticulous cleaning process, beginning with washing using detergent, then rinsing with distilled water, drying, and final sterilization in a hot air oven at 121°C for 2 h. To maintain sterility, each piece of glassware was wrapped with aluminum foil before sterilising. Furthermore, the wire loops utilized for inoculation were disinfected by soaking them in 70% alcohol and then subjected to flames from a spirit lamp.

Furthermore, strict hygiene measures were observed to maintain a clean laboratory environment. Before and after each laboratory session, the workbench was thoroughly swabbed with 95% ethanol to ensure effective disinfection and prevent cross-contamination. These meticulous procedures were implemented to minimize the risk of contamination and maintain the integrity of the experimental setup throughout the research process.

2.3 SAMPLE COLLECTION

Sterile cotton swab sticks were employed to meticulously gather samples from the surfaces of computer keyboards located in designated offices within the institution, including the Registrar's office, the Deputy Vice-Chancellor's office, the Vice-Chancellor's office, and selected faculties. All sampling procedures were conducted under aseptic conditions to prevent contamination.

Before sampling, both the swab sticks and sterile media were appropriately labelled to ensure the accurate identification of samples. To collect samples, one sterile swab stick wet with sterile physiological saline was utilized to swab the surface of the keyboard buttons. Additionally, the palms of the users were swabbed using sterile swab sticks in an aseptic fashion.

To maintain sample integrity during transportation, both the swab sticks and sterile media were carefully placed on an ice pack medium before being transported to the laboratory for microbial investigation. These meticulous sampling techniques were employed to verify the dependability and precision of the microbiological study done on the sample surfaces.

2.4 ISOLATION OF MICROORGANISMS

The approach given by Okoro et al. [6] was applied in this investigation. At first, the swab stick with the sample was immersed in a test tube containing sterile normal saline and stirred for a length of 5 mins. Subsequently, inoculations were

recovered from the saline solution and cultivated using both streak and spread plate procedures. The inoculum was extracted aseptically in duplicates from the normal saline solution for every sample. One the inoculum was then evenly dispersed across the surface of a firm, sterile nutrient agar plate using a flamed wire loop. Additionally, the same technique was replicated for solid Sabouraud Dextrose Agar, which is specifically developed for fungal cultivation. Subsequently, the inoculation plates were incubated under proper conditions. Bacterial culture plates were kept at a temperature of 37 °C for a period ranging from 24 to 48 h, whilst fungal culture plates were permitted to incubate at room temperature for a length of 2 to 5 days. Throughout the incubation phase, the plates were checked daily for any growth signs. Upon the detection of growth, each culture plate underwent analysis to identify unique colonies. Sub-cultures were then produced from these colonies on fresh solid agar media and subjected to further incubation as previously reported. The subsequent growth was carefully studied for homogeneity, providing an indicator of purity. Once pure cultures were obtained, they were utilized for characterization and eventual identification. The culture media employed in this method included nutritional agar, utilized for the growing of non-fastidious organisms, and Sabouraud Dextrose Agar, which was specially chosen for fungal cultivation. This careful methodology ensured the systematic isolation, cultivation, and identification of microorganisms present on the collected surfaces, offering vital insights into the microbial landscape of the computer keyboards within the specified offices.

2.5 ENUMERATION OF MICROORGANISMS

Colonies that developed were enumerated and recorded as colony-forming units (CFU). The colony counter was utilized to obtain the total number of colony-forming units per millimeter.

2.6 IDENTIFICATION OF ISOLATES

Representative isolated colonies underwent purification through sub-culturing on specific culture media plates at suitable temperatures. Post-incubation, discernible colonies on agar plates were meticulously chosen and inoculated onto agar slants. These slants have been chilled and later exploited for additional studies, as stated by Mehmet et al. [7]. Bacterial isolates were characterized using a blend of colonial, microscopic, and biochemical traits. Their traits were examined, and cross-referenced with established taxa in standard manuals. Bergey's Manual of Determinative Bacteriology was consulted for bacterial identification, with isolates named according to their species. Likewise, fungi isolated from Sabouraud Dextrose Agar media plates underwent characterization and identification primarily based on colony features and microscopic examinations. The colonies were scrutinized for fungal mycelia presence, septate or non-septate mycelia determination, conidia presence, spore colouration (e.g., black or green), and conidiophore arrangement. Lactophenol cotton blue mounts of fungal isolates were prepared for microscopic examination, individually scrutinized under 10X and 40X objective lenses. These observations were cross-referenced with mycological atlas materials to ensure precise fungal species identification [8]. This meticulous approach guaranteed thorough characterization and accurate identification of bacterial and fungal isolates from the sampled computer keyboard surfaces.

2.7 STANDARDIZATION OF INOCULUM

In the process of standardizing test organisms, a 0.5 McFarland scale was employed. This scale ensures consistency in the concentration of test organisms by providing a turbidity standard equivalent to an optical density of 0.08-0.13 at a wavelength of 625 nm. This optical density corresponds to the density of a bacterial culture containing 1 x 108 colony-forming units (CFU/ml) [9-12]. This standardized approach ensures uniformity and accuracy in the testing process, enabling reliable and reproducible results across experiments.

2.8 ANTIBIOTIC SUSCEPTIBILITY TESTING

The isolates were subjected to antibacterial susceptibility testing using the disc diffusion method. A Muller-Hinton agar medium was made and placed into sterile plates, where it solidified at the ambient temperature. Afterwards, several colonies from the newly grown culture of the isolates were spread onto the dry agar plates.

Several antibiotic discs, each containing a distinct antibiotic (such as Reflacine, Augmentin, Nalidixic acid, Ciproflox, Gentamycin, Streptomycin, Septrin, Ceporex, Ofloxacin, and Ampicillin) were carefully and firmly inserted onto the agar plates using sterile forceps. The plates were thereafter placed in an incubator and maintained at a temperature range of 35-37 °C for 18-24 h.

Following incubation, the antibiotic discs were surrounded by zones of inhibition, which were then inspected and measured to the nearest millimetre. The results were reported accordingly. The isolates were categorized as resistant, intermediate, or sensitive according to the categories outlined by the Clinical and Laboratory Standard Institute and the parameters set by the World Health Organisation (WHO).

Furthermore, an isolate was categorized as multi-drug resistant if it demonstrated resistance to at least three of the antibiotics tested. This comprehensive approach allowed for assessing the isolates' susceptibility to various antibiotics, providing valuable insights into their resistance profiles and facilitating informed decision-making regarding antibiotic treatment strategies.

2.9 MOLECULAR CHARACTERIZATION OF ISOLATES

The Zymo Research Quick-DNA Fungal/Bacterial Miniprep kit served as a pivotal tool in the purification of genomic DNA extracted from bacterial isolates. This kit is highly versatile, capable of isolating DNA from a diverse array of tough-to-lyse fungi and bacteria, including notable species such as *C. albicans, A. fumigatus, N. crassa, S. cerevisiae, S. pombe*, as well as from mycelium and both Gram-positive and Gram-negative bacterial strains [13]. The kit's utilization of ultra-high density Bashing Beads enables rapid and efficient lysis of cellular membranes without the necessity for organic denaturants or proteinases. This results in the extraction of

high-quality genomic DNA, which is crucial for downstream molecular applications such as PCR and array analysis.

To amplify the 16S rRNA genes, PCR (Polymerase Chain Reaction) was employed, utilizing a set of universal primers. The reaction mixture comprised One Taq Quick load 2X Master Mix, the extracted DNA template, and nuclease-free water. After thirty repetitions of denaturation, annealing, and elongation, the heat cycling protocols involved an initial denaturation step. This was then followed by a final extension step to guarantee the DNA synthesis was fully completed.

Following PCR amplification, the resultant amplicons underwent purification to remove any remaining impurities or contaminants. This purification step is crucial for ensuring the fidelity of subsequent analyses.

Gel electrophoresis was then employed to separate and visualize the amplified DNA fragments. The expected size of the 16S rRNA gene amplicons was approximately 1,550 base pairs. PCR products were mixed with loading buffer and electrophoresed on a 2.0% agarose gel stained with Safeview™ Classics. DNA ladder controls were included to facilitate accurate size determination. Electrophoresis was conducted at a constant voltage for a predetermined duration to ensure optimal separation of DNA fragments based on size.

Subsequently, the purified PCR products were sent to Inqaba Biotech, South Africa, for Sanger sequencing using an Applied Biosystems automatic DNA sequencer and BigDye kit. Raw sequences obtained from the sequencer were cleaned using molecular evolutionary genetics analysis (MEGA) software to eliminate any sequencing artifacts or errors.

The cleaned sequences were then subjected to a Blastn search against the NCBI nucleotide database to identify 16S rRNA gene sequences. Percentage similarities between the obtained sequences and those in the database were noted. Phylogenetic trees were then generated using BLAST pairwise alignment, allowing for the accurate identification and phylogenetic analysis of the bacterial isolates. This comprehensive approach provided valuable insights into bacterial isolates' genetic composition and evolutionary relationships, furthering our understanding of microbial diversity and dynamics.

2.10 DATA ANALYSIS

The statistical assessment for this research was conducted using the SPSS version 20.0 program. The results were summarized and presented using descriptive statistics, enabling a clear comprehension of the data's central tendencies and variations. Moreover, inferential statistics, such as the Chi-squared test, were employed to identify significant differences or associations among the study variables. A significance threshold of 0.05 was adopted, indicating that findings with a p-value below 0.05 were deemed statistically significant. This meticulous statistical methodology ensured the precision and dependability of the study's findings, offering valuable insights into the observed data relationships and patterns.

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3. RESULTS

3.1 CULTURAL, MORPHOLOGICAL, AND BIOCHEMICAL CHARACTERISTICS OF ISOLATED MICROORGANISMS.

The findings presented in Table 1 indicate that various microbes, including both harmful and harmless ones, were found to colonize the surfaces of the computer keyboards examined in this study. Among the identified microorganisms were several bacterial species, including *Salmonella* sp., *Bacillus cereus*, *Enterobacter* sp. coagulase-negative *Staphylococcus* sp., *Escherichia coli*, and *Staphylococcus aureus*. This suggests that computer keyboards can harbor potentially harmful bacteria alongside non-pathogenic ones, highlighting the importance of regular cleaning and disinfection to mitigate the risk of microbial contamination. The organisms' cultural characteristics were filamentous, dry, smooth, flat, rhizoid, undulate or raised. It shows that there were three (3) gram-positive and gram-negative organisms respectively. Most of which are rods and cocci respectively. Furthermore, the table shows the biochemical characteristics and identities of isolated microorganisms. The following tests were carried out citrate, oxidase, coagulase, catalase, and indole. The results turned out to be either negative or positive.

3.2 CULTURAL AND MORPHOLOGICAL CHARACTERISTICS OF FUNGAL ISOLATES

Table 1 also presents the various fungi types identified, encompassing *Penicillium* sp., *Aspergillus* sp., and *Mucor* sp., all originating from computer keyboards in certain office environments. It was also seen that all fungi isolates were seen to have a fluffy surface and a septate hypha.

TABLE 1
FREQUENCY OF OCCURRENCES OF THE ISOLATED MICROORGANISMS

Bacterial isolates	No.	%	Fungal isolates	No.	%
Escherichia coli	47	47.4	*Aspergillius* sp	1	33.3
Bacillus sp	15	15.1	*Mucor* sp	1	33.3
Salmonella sp	17	17.1	*Penicillium* sp	1	33.3
Staphylococcus sp	8	8.0			
Staphylococcus sp	7	7.0			
Enterobacter sp	5	5.0			
TOTAL	**99**	**100**	**TOTAL**	**3**	**100**

3.2.1 FREQUENCY OF OCCURRENCE OF THE ISOLATED MICROORGANISMS.

Table 1 also provides an overview of individual bacterial species' occurrence rates on the investigated computer keyboards. *Staphylococcus aureus* has the highest occurrence (47. 4%) followed by *Escherichia coli* (15.1%) and *Salmonella* sp (17.1%), *Staphylococcus* sp (8.0%), *Enterobacter* sp (5.0%) and Coagulase negative

TABLE 2
QUANTITATIVE RESULTS ON DNA PURITY ON THE ISOLATES OBTAINED FROM COMPUTER KEYBOARDS AT SOME OFFICES AT MADONNA UNIVERSITY.

Isolates	DNA Concentration (µg)	Absorbance ratio	A260	A280
10	103.7	1.83	2.07	1.13
11	148.5	1.51	2.97	1.97
12	52.8	1.42	1.06	0.74

KEY: 10: *Bacillus cereus*, 11: *Salmonella* sp, 12: *Enterobacter* sp

your isolates are. An absorbance of 260/280 when you have it 1.8-2.0 indicates that the isolates are very pure; those with 1.6-1.7 are equally pure too. Isolate no. 11 had the highest DNA concentration (148.5 µg) and an absorbance ratio of 1.51 which signifies that it was not pure, this was followed by isolate no. 10 (103.7 µg) with an absorbance ratio of 1.83 which shows it was pure, and isolate no. 12 had the least concentration (52.8 µg) with an absorbance ration of 1.42 signifying a not pure isolate.

Staphylococcus sp (7.0%) respectively *Penicillium* sp, *Aspergillus* sp and *Mucor* sp. were the microbes identified and its frequency of occurrence were 33.3% respectively.

TABLE 3
ANTIBIOTICS SUSCEPTIBILITY TESTING PROFILE AND ITS INTERPRETATION

Identified Organisms	NA	PEF	CN	AU	CPX	SXT	S	PN	CEP	OFX
Escherichia coli	0.0(R)	0.0(R)	0.0(R)	0.0(R)	13.0(R)	0.0(R)	17.0(S)	13.0(R)	0.0(R)	0.0(R)
Bacillus cereus	11.0(R)	25.0(S)	23.0(S)	19.0(S)	21.0(I)	15.0(I)	21.0(S)	21.0(S)	25.0(S)	25.0(S)
Salmonella sp	0.0(R)	0.0(R)	0.0(R)	0.0(R)	27.0(I)	9.0(R)	0.0(R)	0.0(R)	0.0(R)	15.0(I)
Staphylococcus aureus	7.0(R)	9.0(R)	7.0(R)	7.0(I)	13.0(R)	0.0(R)	19.0(S)	11.0(R)	19.0(R)	0.0(R)
Staphylococcus sp	0.0(R)	15.0(S)	9.0(R)	9.0(R)	23.0(I)	15.0(I)	15.0(S)	15.0(I)	17.0(I)	21.0(S)
Enterobacter sp	9.0(R)	11.0(I)	0.0(R)	11.0(R)	25.0(I)	0.0(R)	0.0(R)	0.0(R)	9.0(I)	19.0(S)

KEY: NA: Nalidixic acid, PEF: Reflacine, GEN: Gentamycin, AUG: Augmentin, CPX: Ciproflox, SXT: Septrin, S: Streptomycin, APM: Ampicillin, CEP: Ceporex, OFX: Ofloxacin, R: Resistance, S: Susceptible, I: Intermediate, Clinical and Laboratory Standard Institute (CLSI) Guidelines 2021: 31st Edition. **CLSI Breakpoints:** S ≥ 20, I = 15- 19, R ≤ 14

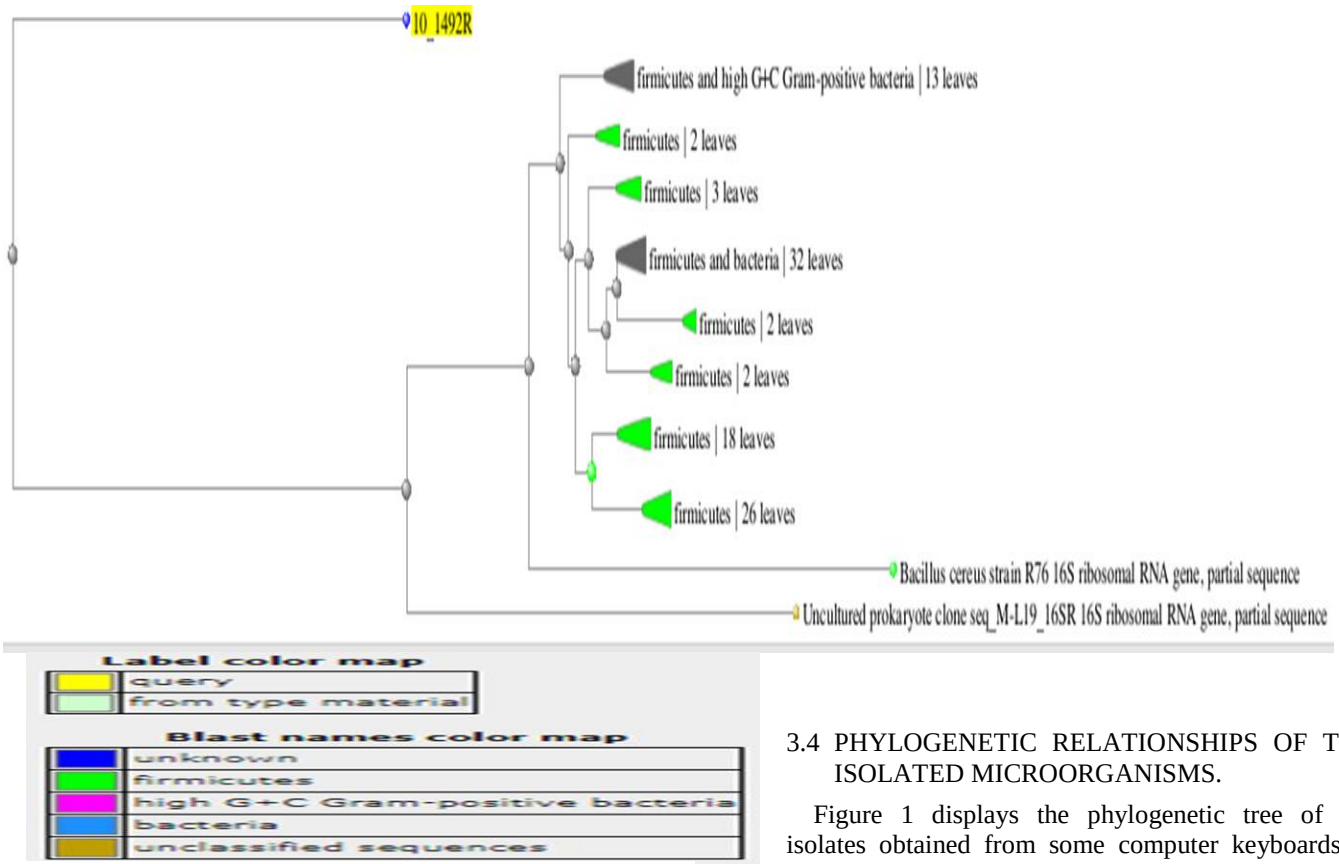

Fig. 1. Phylogenetic Tree for Bacillus cereus using pairwise alignments

3.3 MOLECULAR CHARACTERISTICS OF THE ISOLATED MICROORGANISMS

Table 2 shows the quantitative results on DNA purity on the isolates obtained from computer keyboards at some offices at Madonna University. A quantified work tells you how pure

3.4 PHYLOGENETIC RELATIONSHIPS OF THE ISOLATED MICROORGANISMS.

Figure 1 displays the phylogenetic tree of the isolates obtained from some computer keyboards at Madonna University. From the tree, it should be that *Bacillus cereus* was identified which shares the same homology with the reference sequences used for the blast.

3.5 ANTIBIOTICS SUSCEPTIBILITY PROFILE OF THE ISOLATED ORGANISMS.

Table 3 exhibits the antibiotic susceptibility patterns of the

isolated organisms. Analysis of Table 2 indicates diverse responses of the isolated bacteria to the antibiotics employed. Table 2 also shows the interpretation of the antibiotics susceptibility profile that shows the susceptible isolates, susceptible to increased exposure and resistance to the different antibiotics used during the study.

4. DISCUSSION

Previous research has highlighted the susceptibility of computer peripherals like keyboards, ATM keypads, and mice to contamination by pathogenic bacteria and fungi [2; 14-17]. This study contributes to this body of knowledge by demonstrating microbial contamination on computer surfaces within a university environment. The findings suggest that the multiple-user setting of universities may facilitate contamination, particularly by bacteria like *Staphylococcus aureus*, which can persist on surfaces for extended periods, potentially acting as vectors for pathogen transmission [18].

The widespread resistance observed among the isolated organisms to antibiotics like Nalidixic acid and gentamicin is of particular concern. Antibiotic-resistant infections pose significant public health risks, increasing treatment failures and the severity of diseases [19]. The ubiquitous presence of *Staphylococcus aureus* in cyber cafés, a known human pathogen, underscores the potential health implications associated with contaminated computer peripherals. Similarly, the isolation of *Staphylococcus epidermidis*, typically a harmless skin inhabitant but capable of opportunistic pathogenicity, raises concern about infection risks [18].

The presence of *Aspergillus* sp. on computer peripherals suggests a potential risk of aspergillosis transmission in cybercafés. The ineffective aseptic practices observed in computer centers underscore the need for improved hygiene protocols to mitigate microbial contamination. Unfortunately, the study reveals a lack of awareness among computer users regarding the health risks associated with contaminated peripherals. This emphasizes the urgent need for education on proper hygiene practices, including avoiding eating while using computers, promoting regular hand washing, and implementing routine disinfection of keyboards and mice to reduce microbial transmission.

Given the omnipresence of microbes in our environment, adopting stringent hand hygiene practices before and after computer use is strongly recommended. Regular cleaning of keyboards and mice with disinfectants is also essential to minimize contamination. The varying levels of contamination observed between cybercafés and office environments highlight the importance of factors like user frequency, cleaning practices, and personal hygiene habits. Enhanced awareness and adherence to hygiene protocols are crucial for mitigating the risk of infection transmission via computer peripherals.

5. CONCLUSION

In this investigation, the presence of *Staphylococcus aureus* in all cybercafés underscores its significance as a medically relevant bacterium capable of causing various human diseases. Conversely, isolating *Staphylococcus epidermidis* from most samples highlights its common occurrence as a commensal organism on human skin. Yet, it can pose a risk of opportunistic infections, such as endocarditis [18]. Moreover, detecting *Aspergillus* sp. in most samples suggests the potential transmission of aspergillosis in cybercafés through contaminated surfaces like computer keyboards and mice.

The identification of these microorganisms on computer peripherals is concerning due to their pathogenic potential and the risk they pose to public health. Therefore, it is imperative to implement proper cleaning and sanitation protocols for computer keyboards to mitigate the spread of infections. Enhancing hygiene practices and routinely disinfecting computer peripherals can help reduce microbial contamination and minimize the risk of infection transmission in environments like cybercafés. These measures are essential for maintaining a safe and hygienic computing environment and safeguarding the health and well-being of users.

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