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Transmission of antibiotic-resistant bacteria through laptop keyboard among students of a tertiary institution in Lagos, Nigeria and the associated risk factors

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Abstract
In today's technology world, the use of the laptop is a global practice. This device could be a means of transmission of antibiotic-resistant bacteria because of its regular use by man. This study therefore aims to determine the bacteria contamination of laptop keyboards with antibiotic-resistant strains among students of a tertiary institution in Lagos, Nigeria. A total of thirty four (34) laptop keyboard swab samples were collected from male and female students respectively. Standard bacteriological identification methods were employed to ascertain the identity of likely contaminants. The culture media used for isolation and identification procedures included: nutrient agar, MacConkey agar, mannitol salt agar and eosin methylene blue agar. Reactions to Gram staining and key biochemical tests were performed using standard protocols. Antibiotic susceptibility testing was done using the disc diffusion method to determine the susceptibility pattern of the isolates followed by extended spectrum beta lactamase (ESBL) phenotypic test on the recovered isolates. A self-structured questionnaire was used to obtain data on laptop usage from each participant. The distribution of the isolates across both gender (Male vs Female) was 1:1 with the isolates: *Staphylococcus aureus* (72%), *Escherichia coli* (16%), *Klebsiella pneumoniae* (10%) and *Shigella* spp. (2%). Multi-drug resistance to *S. aureus, K. pneumoniae* and *E. coli* with *S. aureus*, resistance to amoxicillin-clavulanic acid (59%), erythromycin (88%), ciprofloxacin (64%), and clotrimazole (52%) were observed. *Klebsiella pneumoniae* demonstrated resistance to amoxicillin-clavulanic acid (82%), ceftazidime (63%), and cefoxitin (55%). Finally, in *E. coli*, resistance to amoxicillin-clavulanic acid, cefoxitin, ciprofloxacin, ceftazidime (100%) and imipenem (50%) was observed. A total of 2 (2.9%) of the isolates were ESBL-positive. Majority 50 (74%) of the students were between the ages of 20 – 24 years. Significant association ($p < 0.05$) was found between colonization of laptop keyboards by resistant isolates and sharing of laptops, use of laptops while eating and use of laptop in public gathering. With the high percentage of resistant isolates from laptop keyboards, good personal hygiene/sanitary measures or limited use of the laptop by students where possible is encouraged.

Keywords: Extended spectrum beta lactamase; laptop keyboards, Students, Antibiotic-resistant bacteria, Risk factors

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INTRODUCTION

Laptop keyboard is a dynamic environment. Due to their daily use by man, bacteria are transferred from body surfaces to the laptop keyboards. Most times, public places such as schools, hospitals and/or recreational centers harbor bacteria most especially antibiotic-resistant bacteria (Ezeanya-Bakpa *et al*., 2023). These bacteria can be deposited on surfaces like laptop keyboards. Studies have reported possible contributing factors for bacterial colonization of laptops: poor hand hygiene, non-disinfected laptop surfaces or other devices thus leading to the spread of bacterial pathogens (Siegmund *et al*., 2010). Behavioural traits such as eating over a laptop keyboard have also been reported as a risk factor for bacterial colonization because food residues on the keyboards could promote the growth of bacteria when moisture is trapped (Olu-Taiwo *et al*., 2021).

There are several studies with reported prevalence of bacteria colonization on laptop keyboards globally. Interestingly, reports from African countries like Ghana, Ethiopia, and Egypt had a 100% prevalence whereas, an Asian country (Pakistan) had a 61.3% prevalence (Alemu *et al*., 2015). In Ghana, Tagoe *et al*. (2011) reported 81.8% of bacteria isolated from laptop keyboards as pathogenic with 100% demonstrated resistance to commonly administered antibiotics such as: penicillin, ampicillin, and cloxacillin. There is paucity of information on bacterial contamination of personal laptop keyboards among students in the tertiary institution most especially, antibiotic-resistant strains from Lagos, Nigeria. However, there is an 80% prevalence report from Nigeria of which *Staphylococcus aureus* (53.6%), *Escherichia coli* (25.11%), and *Klebsiella spp*. (14.5%) were the reported isolated bacteria from electronic personal devices (Kawa, 2013).

Globally, there is limited emphasis on laptops as a threat to infection control in schools especially at the tertiary level. As a personal device, with daily contact with bodily surfaces, implementation of infection control practice is highly required. Recent studies have shown that inadequate disinfection of personal devices like laptop accessories, mobile phones can pose health hazard to university students (Bodena *et al*., 2019; Ezeanya-Bakpa *et al*., 2022). Hence, this study was conducted to evaluate the bacterial contamination of laptop keyboards of students in a tertiary institution, identify antibiotic-resistant isolates as well as determine possible risk factors.

MATERIALS AND METHODS

Sample Collection

Moist sterile cotton swab stick was gently rolled over the surfaces of 68 randomly selected laptop keyboards- 34 from each gender across departments in the university. The sterile cotton swab sticks were moistened in 1% normal saline. Swabs from the keyboard was carried out by thoroughly wiping out the space between individual keys. Prior to sample collection, the students completed a self-structured questionnaire on their usage of laptop and other related data was collected. Informed consent was given by the students prior to sample collection which allowed the sampling of the laptop keyboards.

Identification of bacterial isolates

The laptop keyboard swabs were inoculated and aerobically incubated for 18 – 24 hours at 37°C. They were inoculated on different growth media such as s nutrient agar, macconkey agar, mannitol salt agar (HIMEDIA Laboratories PVT Ltd, Mumbai, Maharashtra, India), eosin methylene blue agar (Biomark Lab, Puna, Maharashtra, India). Pure culture of the isolates was obtained by sub-culturing discrete colonies on a fresh nutrient agar plate. All bacterial isolates were presumptively identified via colonial morphology, and gram staining. The isolates were further confirmed using biochemical tests- catalase, motility, urease, sugar fermentation, indole and citrate tests as recommended by Wanger et al. (2017).

Antimicrobial Susceptibility Testing of isolates

The modified Kirby-Bauer disc diffusion method was used for the antimicrobial susceptibility testing in triplicates. The Muller Hinton agar (Oxoid Cambridge, UK) was aseptically mounted with the antibiotic discs proceeding the inoculation with the test organisms. The standard turbidity of the inoculum was equivalent to 0.5 McFarland standard using a spectrophotometer.

The susceptibility pattern – susceptible, intermediate and resistance of each bacterial isolate was analysed following the production of zone of inhibition in accordance with the Clinical and Laboratory Standards Institute's standard criteria (CLSI, 2020). The antibiotics tested included: ciprofloxacin (5µg), tetracycline (30µg), gentamicin (10µg), cefoxitin (30µg), ceftazidime (30µg), cotrimoxazole (50µg), erythromycin (15µg), amoxicillin-clavulanic acid (30µg), ceftriaxone (30µg), cefotaxime (30µg) and imipenem (10µg) (Liofichem s.r.l, Italy).

Phenotypic resistance test - Double disc synergy test

Detection of extended-spectrum beta-lactamase isolates was done using the double disc synergy test according to methods described by Ezeanya *et al.* (2017). The inoculum suspension of the test isolates was prepared to turbidity of 0.5 McFarland equivalent prior to inoculation on Mueller-Hinton agar plate. Afterwards, the inoculated plates were incubated at 37°C aerobically for 24 – 48 hours. Clear dome-shaped zone of cephalosporin/clavulanate synergy was observed and interpreted as appropriate. Test controls were used as recommended by the clinical and laboratory standards institute's standard criteria (CLSI, 2020).

Data analysis

Descriptive statistics was used to represent number and percentage where applicable. Chi-square was used to establish the relationship between predisposing factors and bacteria isolated from the laptop keyboards. Significant relationship was established when *P*-value < 0.05.

RESULTS

Prevalence of bacteria and possible risk factors

The prevalence of bacteria on the laptop keyboards was 44.1% (30/68). The percentage occurrence of isolated bacteria from laptop keyboard swabs were as follows: *Staphylococcus aureus* (72% (22/30), *Escherichia coli* (16% (4/30), *Klebsiella pneumoniae* (10% (3/30) and *Shigella spp*. (2% (1/30).

The age distribution of the students was 15 – 19 years 16 (23%), 20 – 24 years 50 (74%) and 25 – 29 years 1 (2%). A statistical significant relationship was found between variables (sharing of laptops, use of laptop while eating and use of laptop in public gathering) and bacterial contamination of laptop keyboards ($p < 0.05$) **(Table 1)**.

Susceptibility pattern of the isolates

All isolated bacteria from laptop keyboards were subjected to antibiotic susceptibility testing against eleven (11) antibiotics in triplicates. The antibiotics with the highest and least activity against the isolates was imipenem (62%) and cefotaxime (8%). High resistance was observed for cefoxitin (80%) and cefotaxime (92%) among bacteria isolated from laptop keyboards swabs respectively **(Figure 1)**. Whereas, the least resistance was observed for tetracycline (25%) and ceftriaxone (31.6%) among bacteria isolated. In this study, *Klebsiella pneumoniae* and *Escherichia coli* had the highest resistance to amoxicillin-clavulanic acid 82% vs. 100%.

Antibiotic-resistant isolates

The prevalence of antibiotic-resistant bacteria was 51.7 %. In this study, *S. aureus* isolates were multi-drug resistant to amoxicillin-clavulanic acid (59%), erythromycin (88%), ciprofloxacin (64%), clotrimazole (52%). *Klebsiella pneumoniae* also demonstrated multi-drug resistance to amoxicillin-clavulanic acid (82%), ceftazidime (63%), cefoxitin (55%). The *E. coli* isolate had 100% resistance to amoxicillin-clavulanic acid, erythromycin, ciprofloxacin, ceftazidime, tetracycline, gentamicin, cefoxitin and clotrimazole. The *S. aureus* isolated from the student's laptop keyboards showed a significant 21 (31%) resistance to Cefoxitin. Production of extended spectrum beta lactamase (ESBL) was observed among *E. coli* isolates 2 (2.9%) recovered in this study.

Table 1: Predisposing factors for the colonization of bacteria on laptop keyboards (n = 68) among tertiary students

Variables	N (%)	N (%) isolated bacteria	P-value
Frequent and constant usage of laptop keyboards	45 (66.2)	29 (69.0)	0.081
Sharing of mobile laptop keyboards	61 (89.5)	33 (78.6)	0.019*
Use of laptop while eating	58 (85.3)	27 (64.3)	0.042*
Use of laptop in public places	63 (92.6)	20 (47.6)	0.028*
Use of laptop when visiting the health center	3 (4.0%)	1 (2.4)	0.077

KEY: *- significant relationship ($p < 0.05$)

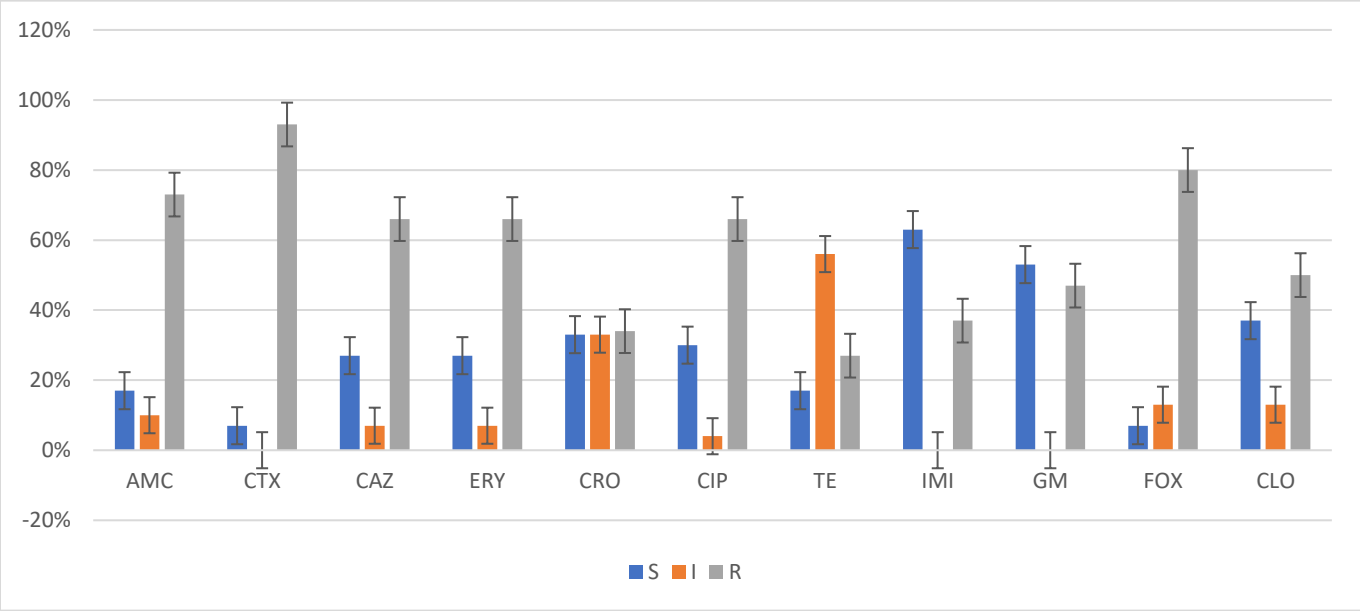

Figure 1: Antimicrobial Susceptibility of the Isolates

Key: AMC: Amoxicillin-clavulanic acid, CTX: Cefotaxime, CAZ: Ceftazidime, ERY: Erythromycin, CRO: Ceftriaxone, CIP: Ciprofloxacin, TE: Tetracycline, IMI: Imipenem, GM: Gentamicin, FOX: Cefoxitin, CLO: Clotrimazole. S - Susceptible, I - Intermediate, R - Resistance

DISCUSSION

This study revealed 44.1% bacterial contamination of the laptop keyboards belonging to students between the ages of 15 – 29 years in a tertiary institution. Our study is in concordance with studies by Olaitan *et al.* (2020) in Lagos State University, Ojo although their study was done in a different location and on 100 desktop computers (not personal laptops) suited at 3 different locations within the university setting. This is the only available data from the South-west region of Nigeria. A significant number of studies have revealed the prevalence of bacterial contamination of laptop keyboards. Study by Nazeri *et al.* (2019) reported a higher contamination of personal computer keyboards of 76% and 99% in Iran and India respectively. Contrary to studies reported from the United States of America and Netherlands with lower rates of 24% and 6.8% respectively (Bures *et al*., 2000). There is paucity of global information on bacterial contamination of laptop keyboards belonging to students; however, this study finding of 44.1% bacterial contamination rate of laptop keyboards is somewhat similar to documented rates of 43.3% reported in Ghana among university students with minimal sharing practice of laptops (Olu-Taiwo *et al*., 2021). These discrepancies could be due to difference in hand washing practice, frequency of disinfecting device, methodology and public knowledge of disease transmission.

In this study, *Staphylococcus aureus* (72%), *Klebsiella pneumoniae* (16%), and *Escherichia coli* (10%) were the predominant bacteria isolated. This corroborates studies in Ethiopia, Nigeria, Iraq and India with prevalence of 14.4%, 20%, 35% and 54.1% for *Staphylococcus aureus* as the most dominant bacteria from students' personal devices such as computer keyboards (Tambe and Pai, 2012; Koscova *et al*., 2018). *Staphylococcus aureus* is a well-known normal flora of the skin and also colonizes the nasal cavity (Akujobi *et al*., 2013). However, this bacterium is notorious for skin and soft tissue infections leading to complicated infections like pneumonia, septicemia and blood infections (Jamalludeen, 2020). Similarly, lower prevalence data for *Klebsiella* spp. have been documented in Ghana and Nigeria of 12.9% and 15.4% respectively (Alemu *et al*., 2015, Olu-Taiwo *et al*., 2021). *Klebsiella* spp. is responsible for hospital and community-acquired infections, with the inclination to disseminate mobile genetic elements (Nwankwo *et al*., 2014). Contrary to this study, higher prevalence of 28.2% have been documented in studies in Iraq (Al-ani *et al*., 2013). The incidence of *Escherichia coli* could be indicative of faecal contamination. Faecal contamination is mostly due to poor sanitary practices. This bacterium is a renowned etiological agent of urinary tract infections, diarrhoea, and sometimes leads to bacteremia (Camins *et al*., 2011). The school environment are well-known for cross-contamination of circulating bacterial strains (Brady and Blair, 2005).

Antibiotic-resistant bacteria continue to thrive as a major global public health challenge with reported high morbidity and mortality, higher treatment rate and prolonged hospital stays (Olu-Taiwo *et al*., 2020). Studies have shown that personal devices such as laptop keyboards and other devices could contribute to the spread of antibiotic-resistant pathogenic bacteria (Verran, 2012; Alemu *et al*., 2015). These antibiotic-resistant bacteria are either multi-drug resistant bacteria or ESBL bacteria as reported in this study. The prevalence (51.7%) of antibiotic resistant bacteria in this study is higher than reported data of 45.1% by Olu-Taiwo *et al.* (2020) among university students in Ghana. The varying prevalence in these countries could be due to antibiotic stewardship practice and also rates of cleaning computer or laptop keyboards. Here, *S. aureus* isolates were multidrug resistant to amoxicillin-clavulanic acid (59%), erythromycin (88%), Ciprofloxacin (64%), clotrimazole (52%). Studies have shown a rise in the prevalence of multi-drug resistant *S. aureus* (Skórczewski *et al*., 2014). *Klebsiella pmeumoniae* was also multi-drug resistant to amoxicillin-clavulanic acid (82%), ceftazidime (63%), cefoxitin (55%). The ESBL producers were 100% *E. coli* isolates which had 100% to amoxicillin-clavulanic acid, erythromycin, ciprofloxacin, ceftazidime, and 50% resistance to imipenem. This supports studies by Akujobi and Ezeanya (2013) with reported carbapenem resistance among ESBL *E.coli* isolates. The *S. aureus* isolated from the student's laptop keyboards showed a significant (31%) resistance to cefoxitin. This isolates are suspected to be methicillin- resistant as cefoxitin is a phenotypic marker for screening methicillin resistance.

Imperatively, effective prevention and control approaches could curtail the spread of these antibiotic-resistant pathogenic bacteria among tertiary students or the general population. The hypothesis that laptop keyboards could be a

reservoir for antibiotic-resistant pathogenic bacteria like multi-drug resistant *Staphylococcus aureus*, *Klebsiella* spp., and third generation cephalosporin resistant (ESBL) *E.coli* among tertiary students was supported by findings from this study.

CONCLUSION

The laptop keyboards of tertiary students were highly contaminated with disease- causing bacteria. These bacteria had varied antibiotic resistance: multidrug resistant and resistance to third generation cephalosporin. Consequently, laptop keyboards could aid the transmission of antibiotic-resistant bacterial pathogens. Therefore, eating while using the laptop, sharing of laptops as well as usage of laptops in public places and/or gathering is strongly discouraged to control the spread of these antibiotic-resistant pathogenic bacteria. This was a single center study. A multi-center longitudinal study will provide robust data that may be useful to policy makers.

Conflict of interest

Authors have no conflict of interest to declare.

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Author contribution

CCE conceived and designed the study, performed data analysis and interpretation and wrote the first draft of the article. MJB collected and assembled the data. All authors approved the manuscripts for publication after reading.

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