Bacteriological Quality of Ready to Eat Vegetable Salads Vended in Ilala District Markets and Antibiotic Sensitivity Profiles of Isolated Contaminant Bacteria

ABSTRACT

Aim: To determine level of bacterial contaminants and antibiotic susceptibility profiles of bacteria isolated from read-to-eat salads (RTES).

Study Design: An experimental cross-sectional study was conducted in three localities of Ilala District in Dar es Salaam (Tanzania).

Methodology: Twenty-four RTES samples were bought from randomly chosen fast food centers. RTE salads were analyzed at the Pharmaceutical Microbiology Laboratory. The total viable counts (TVC) were determined, and the standard procedures for microbial identification were performed and confirmed by physiological tests. The identified microbial contaminants were subjected to antibiotic sensitivity testing (AST) using the Kirby-Bauer disc diffusion method. Six widely used antibiotics: amoxicillin (AX25), sulfamethoxazole/ trimethoprim-(SXT-25), amoxicillin/clavulanic acid (AMC30), gentamicin (CN5), ciprofloxacin (C5), and chloramphenicol (C30) were used for the AST.

Results: The TVC of isolated bacterial contaminants ranged from 10^6 to 10^8 cfu/g, which was above the acceptable standard limit and unfit for human consumption. Five bacterial species comprised of *Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus, Pseudomonas aeruginosa*, and *Klebsiella oxytoca* were isolated and subjected to the AST. All bacteria were resistant to AMC30. *Escherichia coli* was isolated from 10% of the RTES. Association between bio-burden and antibiotic resistance was observed.

Conclusion: The RTES harbored contaminant bacteria beyond acceptable limits. The predominant contaminants were *P. aeruginosa* and *E. coli*. One-fifth of the samples contained *E. coli,* indication of poor sanitation. All the isolated bacteria were resistant to AMC30. Prompt measures are required to curb the spread of antibiotic-resistant microorganisms.

Keywords: [Bacteriological quality, contaminant bacteria, antibiotic sensitivity testing]

1. INTRODUCTION

Ready-to-eat vegetable salads (RTES) are commonly prepared at home and food vendor outlets in several localities of Dar es Salaam, Tanzania. The most common ingredients for RTES include tomatoes, onions, ~~or~~ cucumbers, carrots, cabbages, salts, and sometimes lemon juice or vinegar [1]. Generally, salads are considered a source of vitamins, minerals, proteins, and other nutrients that are important for the proper body functioning and disease fighting [2, 3]. Since

RTES are consumed without cooking [4]. They are considered potential sources of enteropathogenic microbes and other food-borne diseases. Bacteria are regarded as the leading source, accounting for a significant 66% of food-borne diseases, followed by chemicals (26%) [5, 6]. The most common enteropathogenic bacterial contaminants in fresh produce include *Campylobacteria* spp., *Salmonella* spp*., Escherichia coli,* and *Shigella* spp. [7], of which some are common causes of food-related illnesses.

On the other hand, the spread of antibiotic resistance through the food chain is also a global health concern [8]. Not only because antibiotics are used in aquaculture, agriculture, and veterinary medicine, but also because antibiotic-resistant bacteria and genes can simply spread through the food chain [8-10]. Currently, different types of antibiotic-resistant microorganisms have been found in both food products and humans [11, 12]. However, basic safety measures such as proper food handling effective cooking may significantly control the dissemination of antibiotic-resistant food-borne pathogens [13-15]. Utilization of pesticides and manures derived from animals irrationally exposed to antibiotics has greatly contributed to the further spread of antibiotic resistance, as some traces of antibiotics remain intact and that are found in the environment such as in soil and water [16, 17].

We therefore aimed to assess the microbiological quality of RTES by isolating the microbial contaminants and determining their antibiotic sensitivity patterns. The findings, thus, can provide an insight into the microbial quality of RTES and their potential role in spreading antibiotic resistance emanating from the contaminant bacteria.

2. MATERIAL AND METHODS

2.1 Study design, areas and samples collection

This was a cross sectional-experimental based study involving the collection of RTES from various fast-food vendors in three localities (Kariakoo, Muhimbili and Buguruni), which are well-known for their commercial activities in Ilala District, Dar es Salaam. The district is one of the populous areas in Dar es Salaam City with over 1.3 (17.6%) million inhabitants. Samples of RTES were bought from randomly selected fast-food vendors between March 2021 and April 2021. About 200 g of RTES mixtures from each vendor which are usually served directly to consumers were aseptically collected into sterile polythene bags, kept in a cool box, maintained at 0–4 °C and aseptically transported to Pharmaceutical Microbiology Laboratory at School of Pharmacy, MUHAS. The collected samples were processed within two hours upon arrival at the laboratory.

Fig. 1. A map of Dar es Salaam and the study area -Ilala District

2.2 Microbial quantification, isolation and identification

Ten (10) grams of each sample were transferred into 90 mL of normal saline and gently agitated for 5 minutes. The supernatant (1 mL) of each content was tenfold serially diluted, from which one aliquot (1 mL) of it was deposited onto

both selective (Mannitol salt agar, Hektoen enteric agar, Mac Conkey agar) and non-selective (Mueller-Hinton, Nutrient, and Sabouraud dextrose) agar plates.

The inoculated plates were then inverted and incubated at 37°C for 24-48 hours. After 24 hours' incubation, all discrete colonies were enumerated, designated as TVC, and expressed in terms of colony-forming units per gram (cfu/gr). Pure colonies were identified through conventional methods such as Gram staining and colony morphology (size, color, form, opacity, and swarming). The characterization of isolates was confirmed biochemically by oxidase, catalase, citrate, urease, sulfide indole motility tests, and triple sugar iron tests [18].

2.3 Antibacterial sensitivity testing

The identified microbial contaminants (bacteria) from RTES were overnight sub-cultured in freshly prepared nutrient broth, and the resultant turbidity was compared to the McFarland 0.5 standard (equivalent to $1.5x10^8$cfu/ml) prior to performing the AST. Then, the bacteria were subjected to AST profiling against six commonly used antibiotics for treatment of bacteria-related infections as per the Standard Treatment Guidelines and National Essential Medicines List [19]: amoxicillin (AX25µg), ciprofloxacin (CIP5µg), gentamicin (CN10µg), sulfamethoxazole/trimethoprim (SXT25µg), chloramphenicol (C30µg), and amoxicillin/clavulanic acid (AMC30µg) (Oxoid, Hampshire, UK). All assays were performed on Mueller-Hinton agar plates (Roth, Germany) using the Kirby-Bauer disk-diffusion method. Following an overnight incubation at 37 °C, the diameters of inhibition zones (IZ) were determined in millimeters. Each of the above tests was performed in triplicate for statistical purposes and reproducibility. Therefore, the resultant IZ was expressed as means and interpreted as per the Clinical Laboratory Standards Institute [20] as sensitive (S), intermediate (I), or resistance (R).

2.4 Statistical Data Analysis

SPSS version 23 was used for analysis, carrying out descriptive statistics (for means and standard deviations) and (ANOVA) of mean microbial counts among RTES from the three localities. Pearson's correlation analysis was also carried out to determine the correlation of bacterial loads among antibiotic-resistant isolates. Differences in IZ between RTES-derived bacteria and the test antibiotics with respect to control bacteria were analyzed by the T-test, and the differences were considered statistically significant at $p<0.05$.

3. RESULTS AND DISCUSSION

The presence of *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Klebsiella* species in RTES is a major health concern. Not only because RTES harbored more than hundred folds the standard limits but also, they are implicated opportunistic bacterial infections [7,11, 12]. Isolation of *Escherichia coli* in 21% (n=5) of the analyzed RTES samples could suggest poor hygienic practices among the street food vendors, and it is a fecal contamination indicator [21, 22]. Since salads are consumed raw, they can act as major vehicles for food-borne illness outbreaks, as has been observed worldwide [7, 11-12, 21]. Generally, raw vegetables, salads included, are capable of supporting bacterial growth due to their high water, neutral pH, and nutrient content. Cross-contamination is one of the main causes of the microbiological contamination of RTES at various points, from farming through preparation to the distribution chain [8, 9], resulting in food-borne disease outbreaks [23, 24]. Therefore, cautious and minimal handling of such produce and cleanliness of equipment and premises are important [11, 13, 16].

In this study, a total of 24 RTES samples were analyzed of which all revealed the presence of contaminant bacteria exceeding acceptable standard limits (**Table 1)**. Our study showed a higher detection of *Pseudomonas aeruginosa* (45.83%; n=11) and *Escherichia coli*, (20.83%' n=5) which differs from other previous studies showing variability in incidences depending on socio- geographic factors [21, 23]. There were statistically significant differences between the established microbiological standard limits and TVC revealed from the tested RTES ($p < .01$).

Table 1. Bacterial contaminants in RTES (expressed as colony forming unit per gram of sample) [25]

Isolated Bacteria	cfu/gr (Minimum-maximum)	Standard limit Cfu/gr	Number of Isolates
Pseudomonas aeruginosa	6.0×10^6- 2.08×10^7	30 to 3×10^2	11
Escherichia coli	4.4×10^6 -1.44×10^7	Absence < 20	5
Staphylococcus aureus	6.0×10^6 - 2.0×10^7	$10^2 < 10^4$	4
Klebsiella pneumoniae	4.0×10^6 - 8.6×10^6	$10^2 < 10^4$	3
Klebsiella oxytoca	7.6×10^6	$10^2 < 10^4$	1

All isolated bacteria were resistant to AMC30, and 66.7% (n=16) to AX25 (**Table 2**). Significant differences between reference microorganisms and the isolated contaminant bacteria against AX25 ($p < .01$; df = 21; 2-tailed) were observed. Six (25%) of 24 isolated bacteria were resistant to C30, and five (20.8%) to SXT25. Of these, four (36.4%) were *P. aeruginosa* and one (*E. coli)* (26%), as shown in **Table 2**.

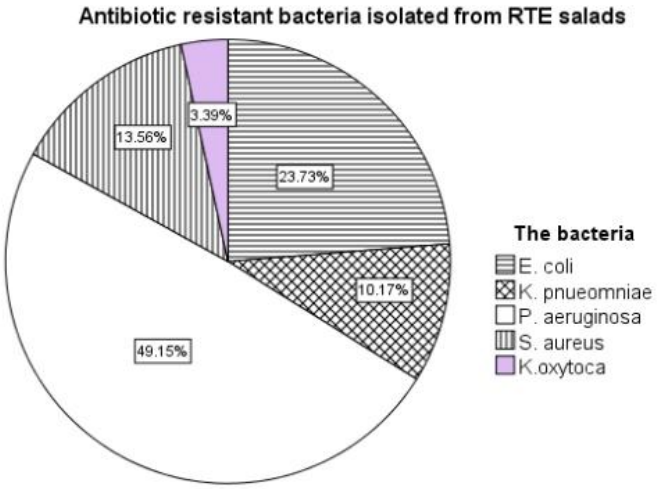
Table 2: Proportions of antibiotic resistant bacteria isolated from RTES

Isolated microbes	Antibiotics (%)					
	AX25	AMC30	C30	SXT25	CIP5	CN10
P. aeruginosa (11)	3(27.3)	11(100.0)	3(27.3)	4(36.4)	-	-
E. coli (5)	5(100.0)	5(100.0)	3(60)	1(20)	-	-
S. aureus (4)	4(100.0)	4(100.0)	-	-	-	-
K. pneumoniae (3)	3(100.0)	3(100.0)	-	-	-	-
K. oxytoca (1)	1(100.0)	1(100.0)	-	-	-	-
Total	16 (66.7)	24(100.0)	6(25.0)	5(20.8)	-	-

Key: (-) means none was resistant to the antibiotic

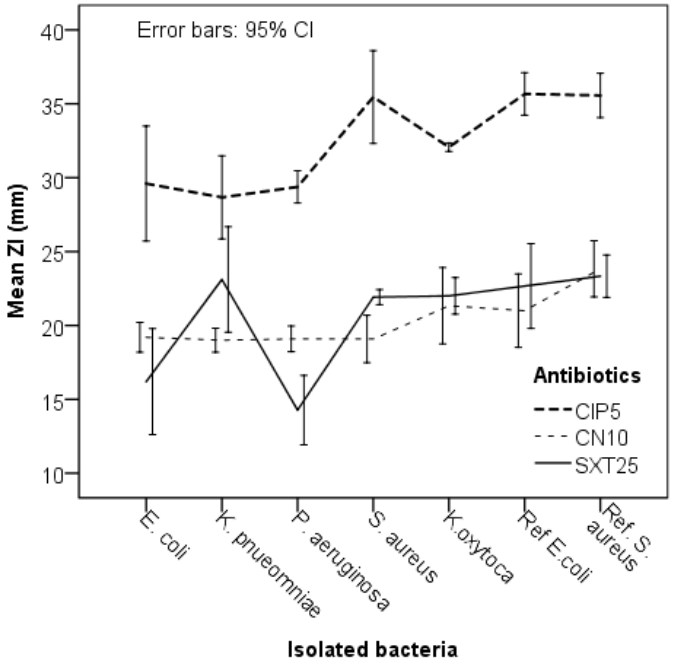
Our study shows that about 49% of the tested RTES-derived bacteria were antibiotic-resistant *P. aeruginosa,* followed *by E. coli* (23.7%), as shown in **Fig. 1**. The incidence of *E. coli* obtained from RTES is slightly lower than what was previously reported (34%) in Pakistan [26] and (8.7%) in Nigeria [22]. *Escherichia coli* is a commensal bacterium; however, some strains have acquired virulence factors and therefore have evolved to pathogenic *E. coli.* When in food, they may cause gastroenteritis and diarrhea [27].

Fig. 1. Proportions of antibiotic resistant bacteria isolated from RTES grouped by species



All tested bacterial isolates were sensitive to CIP5 and CN10 (**Fig. 2** and **Table 2**). This observation was confirmed statistically by both the RTES-derived bacteria and the reference bacteria/control, yielding no significant differences in ZI (p = .51). The T-test (independent samples) analysis of the antibiotic sensitivity of the contaminant bacteria with respect to their respective control organisms revealed different patterns: Significant differences in susceptibility against AMC30, CN10 (p <.001), and SXT25 (p =.014) when *S. aureus* was compared to control organisms. *Klebsiella oxytoca* exhibited a significant difference (p <.001) against CIP5, C30, AMC30, and AX25, in respect to the control organism (**Fig. 2** & **Fig. 3**). Significant differences in sensitivity between control organisms and *P. aeruginosa* were also observed against AMC30, AX25, and CIP4 (p<.001). For *K. pneumonia*e, there were significant differences against C30, AMC30, and AX25. While *E. coli* demonstrated a significant difference against AMC30 and AX25 (p <.001), C30 (p =.038), and CN10 (p =.023).

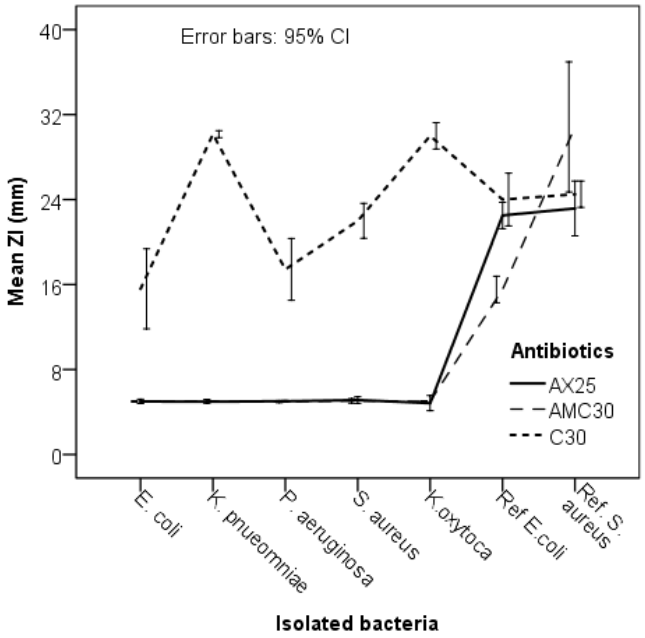
Fig.2. Antibiotic susceptibility patterns of the isolated contaminant bacteria against CIP5, CN10 and SXT25.



A multi-resistant *P. aeruginosa* was isolated in one sample that exerted resistance against three antibiotics, namely SXT25, as depicted in **Fig. 2** above, as well as against AX25 and AMC30 (**Fig. 3)**. This observation could be attributed to prior exposure of the bacteria to the antibiotics, which is largely caused by irrational use of antibiotics [28]. The association between bio-burden (TVC) and prevalence rates of antibiotic resistance was relatively higher among the

Gram-negative bacteria (*P. aeruginosa*, *E. coli, K. pneumoniae,* and *K. oxytoca*) (Pearson's Chi-square = 231.94; $p < .01$). While such an association was observed among isolates of *S. aureus* (Gram-positive bacterial contaminants) against AX25 and AMC30 (Pearson's Chi-square = 299.05; $p < .01$) to which they were resistant to the two antibiotics (**Table 2**).

Fig. 3. Antibiotic sensitivity patterns of isolated contaminant bacteria to AX25, ACM30 and C30.



Staphylococcus aureus is a pathogenic bacterium that causes staphylococcal food poisoning along with other illnesses. Human-origin isolates of *Staphylococcus* are the main source of food poisoning outbreaks in the country [29, 30], where about 1% of deaths annually are attributed to food-related contamination by *S. aureus* [32-30]. As little as 100 thousand *S. aureus* in food may have detrimental effects [31-32]. *Pseudomonas aeruginosa* is an opportunistic pathogen widely distributed in soil, water, and other moist environments. The presence of *P. aeruginosa* in the food sample indicates defective decontamination during preparation [33]. Consumption of contaminated RTES with such a pathogen may have serious human health consequences, particularly for children, elders, and immunologically compromised persons [5,34].

Klebsiella genus is a common opportunistic pathogen for humans and other animals, as well as being transient flora (especially in the gastrointestinal tract). Other habitats include sewage, drinking water, soils, and surface waters [34]. *Klebsiella pneumoniae* is one of the most important members of the *Klebsiella* genus in the Enterobacteriaceae family, known to cause several infections in the upper respiratory and gastrointestinal tracts. The infections are more serious among immunocompromised individuals [35]. It has been reported that clinically relevant features of *K. pneumoniae* may be preserved in wastewater, even after treatment. This evidence highlights the potential of *K. pneumoniae* for spreading through wastewater, enhancing the risks of transmission back to humans [36].

Klebsiella oxytoca is rising as a significant opportunistic pathogen, causing health care facility-acquired infections in neonates as well as adults. The bacterium is responsible for a wide range of ailments, from colitis to infective endocarditis, other than the common urinary and respiratory tract infections [37]. Our study shows that *Klebsiella oxytoca* was resistant to AX25 and AMC30 (**Table 2**). This implies that these antibiotics may not be useful to individuals infected with *Klebsiella oxytoca,* particularly those with weakened immune systems. More importantly, it is also clear now that RTES harbors microorganisms that may carry various antibiotic resistance genes that can be vertically or horizontally transmitted [38].

4. CONCLUSION

The microbial quality of RTES available in the surveyed areas raises health concerns. All tested samples had bacterial loads beyond permissive levels. *Pseudomonas aeruginosa* and *E. coli* were the most abundantly isolated contaminants. The presence of *E. coli* is an indication of poor hygienic practices and insanitary conditions. All the isolated bacteria were resistant to AX25 and AMC30. An association between bacterial load and antibiotic resistance among microbial contaminants was observed.

The authors hypothesize that improper handling of the RTES ingredients and cross-contamination of vegetables during pre- and post-harvesting processes could have been attributed to the poor microbial quality. They recommend that responsible authorities establish more stringent measures stringent measures such as proper hygiene practices and sanitary conditions for RTES vendors to ensure RTES are not the cause of food-borne outbreaks in our community and vehicles of dissemination of antibiotic-resistant pathogens.

5. LIMITATION OF THIS STUDY

Further studies employing a larger sample size and covering wider study areas need to be conducted to ascertain the microbial quality of RTES and the magnitude of resistant pathogens emanating from RTES. Additionally, more research on RTES is needed to isolate certain pathogenic strains of the bacteria found in this study.

ACKNOWLEDGMENTS

The authors extend their gratitude to the Ministry of Education and Vocational Training of Tanzania through the Higher Education Students Loan Board for financial support. They acknowledge the technical support received from the Pharmaceutical Microbiology Laboratory of the School of Pharmacy. Most importantly, they extend their gratitude to the RTES vendors and local government authorities for their permission to conduct this study.

COMPETING INTERESTS

''Authors have declared that no competing interests exist''.

AUTHORS' CONTRIBUTIONS

'Mwambete' designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. 'Naftali' did the data collection, laboratory analysis and managed the literature searches. Both authors read and approved the final manuscript.

ETHICAL APPROVAL

The study was approved by both the School of Pharmacy Research Project Task force and the University Research Ethical Committee prior obtaining permission from the local authorities to conduct the study.

REFERENCES

1. Mbae KM, Ndwiga MK, Kiruki FG. Microbiological Quality of Kachumbari, a Raw Vegetable Salad Popularly Served Alongside Roast Meat in Kenya. *J. Food Qual.* 2018:1–5. DOI: https://doi.org/10.1155/2018/8539029
2. Sarkar T, Salauddin M, Roy S, Chakraborty R, Rebezov M, Shariati MA, Thiruvengadam M, Rengasamy KR. Underutilized green leafy vegetables: Frontier in fortified food development and nutrition. *Rev. Food Sci. Nutr.* 2022 Jun 29:1-55. DOI: 10.1080/10408398.2022.2095555
3. Sharma RK, Coniglio MA, Laganà P. Natural Inflammatory Molecules in Fruits and Vegetables. Berlin/Heidelberg, Germany: Springer; 2022. ISBN: 978-3-030-88472-7
4. Arienzo A, Murgia L, Fraudentali I, Gallo V, Angelini R, Antonini G. Salads during Shelf-Life and Home-Refrigeration. Foods. 2020 Oct 8;9(10):1421. DOI: https://doi.org/10.3390/foods9101421
5. Addis M., Sisay D. A review on major food borne bacterial illnesses. *J. Trop. Dis.* 2015;3(4):1–7.DOI: 10.4176/2329-891X.1000176
6. Guptaa AK, Chaudharyb A. Food Poisoning: causes, its effects and control. INWASCON Technology Magazine (i-TECH MAG). 2022; 4:42-8. DOI: http://doi.org/10.26480/itechmag.04.2022.59.61.
7. Łepecka A, Zielińska D, Szymański P, Buras I, Kołożyn-Krajewska D. Assessment of the Microbiological Quality of Ready-to-Eat Salads—Are There Any Reasons for Concern about Public Health? *Int. J. Environ. Res. Public Health.* 2022 Jan 29;19(3):1582. DOI: https://doi.org/10.3390/ijerph19031582
8. Bester LA, Essack SY. Antibiotic resistance via the food chain: Fact or fiction? *S. Afr. J. Sci.* 2010 Sep 1;106(9):1-5. DOI:10.4102/sajs. v106i9/10.281
9. Sagar P, Azeem A, Banjara SK, Veleri S. The role of food chain in antimicrobial resistance spread and One Health approach to reduce risks. *Int. J. Food Microbiol.* 2023 Feb 24:110148. DOI: https://doi.org/10.1016/j.ijfoodmicro.2023.110148

10. Riwu KH, Effendi MH, Rantam FA, Khairullah AR, Widodo A. A review: Virulence factors of *Klebsiella pneumonia* as emerging infection on the food chain. *Vet. World*. 2022 Sep 1;15(9). DOI: 10.14202/vetworld.2022.2172-2179
11. Ma F, Xu S, Tang Z, Li Z, Zhang L. Use of antimicrobials in food animals and impact of transmission of antimicrobial resistance on humans. *Bio-saf. Health.* 2021, 1;3(1):32-8. DOI: https://doi.org/10.1016/j.bsheal.2020.09.004
12. Abebe E, Gugsa G, Ahmed M. Review on major food-borne zoonotic bacterial pathogens. *J. Trop. Med.* 2020 Oct; 2020.DOI: https://doi.org/10.1155/2020/4674235
13. Caniça M, Manageiro V, Abriouel H, Moran-Gilad J, Franz CM. Antibiotic resistance in food-borne bacteria. *Trends Food Sci Technol.* 2019 Feb 1; 84:41-4. DOI: https://doi.org/10.1016/j.tifs.2018.08.001
14. Abakari G, Cobbina SJ, Yeleliere E. Microbial quality of ready-to-eat vegetable salads vended in the central business district of Tamale, Ghana. *Int J food Contam*. 2018;1–8. DOI: https://doi.org/10.1186/s40550-018-0065-2
15. Kapaya F, Mwansa FD, Sakubita P, Gama A, Langa N, Chewe O, et al. A foodborne disease outbreak investigation experience in a college in Lusaka, Zambia, 2017. *Pan Afr Med J.* 2018;29 (February). DOI:10.11604/pamj.2018.29.100.14737
16. Iwu CD, Korsten L, Okoh AI. The incidence of antibiotic resistance within and beyond the agricultural ecosystem: A concern for public health. *MicrobiologyOpen*.2020;(January):1–28. doi: 10.1002/mbo3.1035
17. Mwambete KD, Stephen WS. Antimicrobial resistance profiles of bacteria isolated from chicken droppings in Dar es Salaam. *Int J Pharm Pharm Sci.* 2015.17(9): 268-271.ISSN- 0975-1491.
18. Cheesbrough M. District laboratory practice in tropical countries, part 2. Cambridge university press; 2005.
19. Standard Treatment Guidelines and National Essential Medicines List in Tanzania (STG/NEMLIT). Accessed on October23,2023 at: https://hssrc.tamisemi.go.tz/storage/app/uploads/public/5ab/e9b/b21/5abe9bb216267130384889.
20. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing, 30th ed. CLSI supplement M100. Clinical and Laboratory Standards Institute, Wayne, PA. 2020.
21. Calonico C, Delfino V, Pesavento G, Mundo M, Nostro AL. Microbiological quality of ready-to-eat salads from processing plant to the consumers. *J. Food Nutr. Res.* 2019; 7:427-34. DOI:10.12691/jfnr-7-6-3
22. Ezemba CC, Mmaduekwe CJ, Udeze CP, Ogumuo FC, Ozoekwe N, Duru BN, Ndulue FK, Okoye PC, Iw U, Ezemba AS, Ezeokoli CM. Antibiogram of *Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Escherichia coli* isolates recovered from ready to eat food samples in ULI campus. International Journal of Frontier Research in Science. 2022;1(1):016-21.
23. Kayombo MC, Mayo AW. Assessment of microbial quality of vegetables irrigated with polluted waters in Dar es Salaam City, Tanzania. *Environ. Ecol. Res.* 2018;6(4):229-39. DOI: 10.13189/eer.2018.060403
24. Possas A, Pérez-Rodríguez F. New insights into Cross-contamination of Fresh-Produce. *Curr. Opin. Food Sci*. 2023 Feb 1; 49:100954. https://doi.org/10.1016/j.cofs.2022.100954
25. Compendium of Microbiological Criteria for Food. Food Standards Australia New Zealand, 2022. ISBN: 978-0-642-34
26. Shah MS, Eppinger M, Ahmed S, Shah AA, Hameed A, Hasan F. Multidrug-resistant diarrheagenic E. coli pathotypes are associated with ready-to-eat salad and vegetables in Pakistan. *J. Korean Soc. Appl. Biol. Chem.* 2015 Apr;58(2):267-73. DOI: https://doi.org/10.1007/s13765-015-0019-9
27. Uzeh RE, Adepoju A. Incidence and survival of *Escherichia coli* O157: H7 and *Listeria monocytogenes* on salad vegetables. *Int. Food Res. J.* 2013 Jul 1;20(4):1921.
28. Olesen SW, Barnett ML, MacFadden DR, Brownstein JS, Hernández-Díaz S, Lipsitch M, Grad YH. The distribution of antibiotic use and its association with antibiotic resistance. *Elife.* 2018 Dec 18;7: e39435. DOI: https://doi.org/10.7554/eLife.39435.001
29. Saifullah S, Abbas F, Samad A, Rizwan M, Bugti FS, Saima R, Yousaf M, Mykhaylo T, Raziq A. 31. *Staphylococcus aureus* prevalence in the fresh salad and vegetables of the Quetta city. *Pure Appl. Biol.* 2018 Mar 2;7(1):255-62. http://dx.doi.org/10.19045/bspab.2018.70031
30. Le HH, Dalsgaard A, Andersen PS, Nguyen HM, Ta YT, Nguyen TT. Large-scale *Staphylococcus aureus* foodborne disease poisoning outbreak among primary school children. *Microbiol. Res.* 2021 Feb 11;12(1):43-52. https://doi.org/10.3390/microbiolres12010005.
31. Abril A, G. Villa T, Barros-Velázquez J, Cañas B, Sánchez-Pérez A, Calo-Mata P, Carrera M. *Staphylococcus aureus* exotoxins and their detection in the dairy industry and mastitis. Toxins. 2020 Aug 20;12(9):537.
32. Liang T, Liang Z, Wu S, Ding Y, Wu Q, Gu B. Global prevalence of *Staphylococcus aureus* in food products and its relationship with the occurrence and development of diabetes mellitus. *Med Adv*. 2023 Mar;1(1):53-78. DOI: https://doi.org/10.1002/med4.6
33. Allydice-Francis K, Brown PD. Diversity of antimicrobial resistance and virulence determinants in *Pseudomonas aeruginosa* associated with fresh vegetables. *Int. J. Microbiol.* 2012 Oct;2012. https://doi.org/10.1155/2012/426241
34. Laborda P, Sanz-García F, Hernando-Amado S, Martínez JL. *Pseudomonas aeruginosa*: An antibiotic resilient pathogen with environmental origin. *Curr. Opin. Microbiol.* 2021 Dec 1; 64:125-32. https://doi.org/10.1016/j.mib.2021.09.010
35. Monteiro A, Cardoso J, Guerra N, Ribeiro E, Viegas C, Cabo Verde S, Sousa-Uva A. Exposure and health effects of bacteria in healthcare units: An overview. *Appl. Sci.* 2022 Feb 13;12(4):1958. https://doi.org/10.3390/app12041958

36. Rocha J, Ferreira C, Mil-Homens D, Busquets A, Fialho AM, Henriques I, Gomila M, Manaia CM. Third generation cephalosporin-resistant *Klebsiella pneumoniae* thriving in patients and in wastewater: what do they have in common? *BMC Genom*. 2022 Dec;23(1):1-4. DOI: https://doi.org/10.1186/s12864-021-08279-6
37. Neog N, Phukan U, Puzari M, Sharma M, Chetia P. *Klebsiella oxytoca* and emerging nosocomial infections. *Curr Microbiol.* 2021 Apr;78(4):1115-23. DOI: https://doi.org/10.1007/s00284-021-02402-2
38. Zhou SY, Wei MY, Giles M, Neilson R, Zheng F, Zhang Q, Zhu YG, Yang XR. Prevalence of antibiotic resistome in ready-to-eat salad. *Front Public Health*. 2020 Mar 25; 8:92. https://DOI.org/10.3389/fpubh.2020.00092