Original Research Article

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

ABSTRACT

Aims: The consumption of ready-to-eat salads (RTES) in fast food outlets has increased as a result of heightened awareness about the nutritional status of these foods and their appeal to consumers looking for healthy and convenient meals. However, microbial contamination during the preparation, transportation, and storage of fresh ingredients has become a public health concern. People who consume RTES are at high risk of contracting food-borne diseases since they are eaten uncooked. Some microbial contaminants in RTES are resistant to antibiotics, increasing the chances of hard-to treat food-borne infections.

Study design: An experimental cross-sectional study was conducted.

Methodology: Twenty-four RTES samples were bought from fast food centers that were chosen at random. The 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 (AX30), 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 microbial contaminants ranged from 10^6 to 10^8 cfu/g, which was unbounded and hence 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 AX30 and 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 AX30 and AMC30. Prompt measures are required to curb the spread of antibiotic-resistant microorganisms.

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

1. INTRODUCTION

Ready-to-eat vegetable salads (RTES) are 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 functioning of the body and skin cancer fighting [2, 3]. Since RTES are consumed uncooked, there is no step to ensure the removal of microbes as compared to cooked food [4]. Hence, RTES are also considered potential sources of enteropathogenic microbes and other food-borne diseases. Bacteria are the second-to-none as the leading source of food-borne diseases (66%), followed by chemicals (26%) [5, 6]. The most common enteropathogenic bacteria contaminants in fresh produce include *Campylobacteria spp.*, *Salmonella spp., Escherichia coli,* and *Shigella spp.* [7]. Such microorganisms are some of the common bacteria that cause food-related illnesses.

On the other hand, the spread of antibiotic resistance through the food chain is another 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 handling of such RTES, effective cooking with adequate temperature, and proper food handling may significantly control the dissemination of antibiotic-resistant food-borne pathogens [13-15]. Utilization of pesticides and manures derived from animals treated with antibiotics has greatly contributed to the further spread of antibiotic resistance, as some traces of antibiotics remain intact [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 collection of RTES from various fast-food vendors in three localities of Ilala District, Dar es Salaam Region. The district is one of the populous areas in Dar es Salaam City with over 1.3 (17.6%) million habitants of Dar es Salaam Region. Samples were randomly bought from fast-food vendors between March 2021 and April 1rom three randomly selected localities namely Kariakoo, Muhimbili and Buguruni. Eight (8) samples were collected from each locality and aseptically transported in cool box to Pharmaceutical Microbiology Laboratory at School of Pharmacy, MUHAS. The collected samples were processed within two hours upon arrival at the laboratory.

2.2 Microbial quantification, isolation and identification

About ten (10) grams of each sample was weighed, and transferred into 90 mL normal saline and gently agitated for 5 minutes. Supernatant of each content was ten-fold serially diluted, from which one aliquot (1 mL) of it was deposited onto selective and non-selective agar plates, and incubated at 37°C for 24-48 hours. After 24 hours' incubation, all discrete colonies were enumerated and designated as TVC and expressed in-term of colony forming unit/gram (cfu/gr). Pure colonies were identified through conventional methods such as colony morphology, Gram staining and biochemical 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 antibiotic sensitivity tests (AST). Then, the bacteria were subjected to AST profiling against six commonly used antibiotics: amoxicillin (AX30µg), 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 [19] as sensitive (S), intermediate (I), or resistance (R).

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3. RESULTS AND DISCUSSION

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The presence of microbial contaminants in RTES is a major health concern. Since salads are consumed raw, they can act as major vehicles for food-borne illness outbreaks, as has been observed worldwide [7, 11-12, 20] Generally, raw vegetables, salads included, are capable of supporting bacterial growth due to their high water, neutral pH, and nutrient content. Hence, 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. All of them revealed the presence of contaminant bacteria that exceeded the allowable limits (**Tables 1** and **2)**. Our study shows a higher incidence of *Pseudomonas aeruginosa* and *Escherichia coli*, which differs from other previous studies showing variability in incidences depending on socio-geographic factors [20, 22]. 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].
During farming or irrigation with microbiologically contaminated water, field-grown vegetables may also become microbiologically contaminated, resulting in food-borne disease outbreaks [22, 23]. Factors such as reduced or enhanced microbial transfer efficiency, cells' adhesion, and the chemical and physical properties of the vegetables and abiotic surfaces have been attributed to the facilitation of the contamination process [23].

Table 1. International Microbiological Criteria [24].

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Microbes	Microbiological Criteria (cfu/gr)	Microbiological Quality
Pseudomonas	$< 10^2$	Satisfactory
	30 to 3 x 10^2	Acceptable
	$> 3 \times 10^2$	Unsatisfactory
E. coli	Absence	Satisfactory
	Presence (<20)	Acceptable
	20 < 10^2	Unsatisfactory
S. aureus	$< 10^2$	Satisfactory
	$10^2 < 10^4$	Acceptable
	$> 10^4$	Unsatisfactory
Enteric coliforms	$< 10^2$	Satisfactory
	$10^2 < 10^4$	Acceptable
	$> 10^4$	Unsatisfactory

Table 2. Bacterial contaminants obtained from RTES (expressed as colony forming unit per each tested gram of sample)

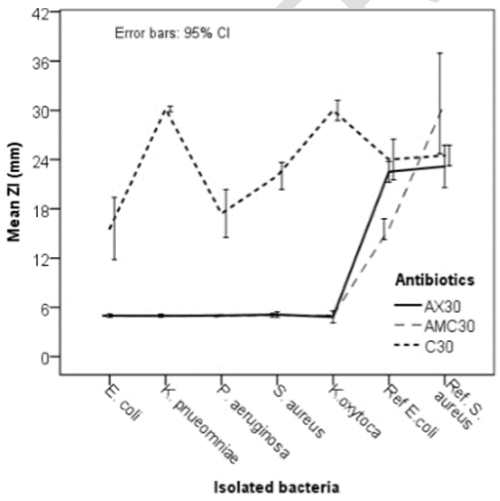
Isolated bacteria	cfu/gr (Minimum-maximum)	Number Isolates/samples
Pseudomonas aeruginosa	6.0 x 10^6- 2.08 x 10^7	11
Escherichia coli	4.4 x 10^6 -1.44 x 10^7	5

Staphylococcus aureus	6.0×10^6 - 2.0×10^7	4
Klebsiella pneumoniae	4.0×10^6 - 8.6×10^6	3
Klebsiella oxytoca	7.6×10^6	1

There were statistically significant differences between the established microbiological limits and TVC revealed from the tested RTES (p< .01). The most frequently isolated contaminant bacteria were *P. aeruginosa* (45.83%; n = 11), while only one isolate of *Klebsiella oxytoca* (4.17%) was found (**Table 2**). All tested bacterial isolates were sensitive to CIP5 and CN10. Except for one isolate of *E. coli* and another of *P. aeruginosa* that exhibited an intermediate susceptibility to CIP5 and CN10, respectively (**Fig. 1**). 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 AX30, in respect to the control organism. Significant differences in sensitivity between control organisms and *P. aeruginosa* were also observed against AMC30, AX30, and CIP4 (p<.001). For *K. pneumonia*e, there were significant differences against C30, AMC30, and AX30. While *E. coli* demonstrated a significant difference against AMC30 and AX30 (p<.001), C30 (p =.038), and CN10 (p =.023).

Fig. 1. Antibiotic sensitivity patterns of isolated contaminant bacteria to ACM30, AX30 and C30.



All isolated bacteria were resistant to AX30 and AMC30 (**Fig. 2**). Significant differences between reference microorganisms and the isolated contaminant bacteria against AX30 (p < .01; df = 21; 2-tailed) were observed. Six (25%) of 24 isolated bacteria were resistant to C30 and five (20.8%) to SXT25, as shown in **Table 3**.

Table 3: Proportions of antibiotic resistant bacteria isolated from RTES

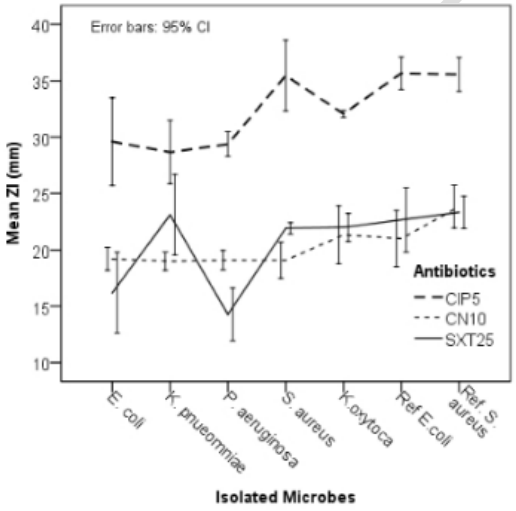
Isolated microbes	**Antibiotics (%)**

	AX30	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	24 (100.0)	24(100.0)	6(25.0)	5(20.8)	-	-

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

A multi-resistant *P. aeruginosa* was isolated in one sample thatexerted resistance against three antibiotics, namely SXT25, as depicted in **Fig. 1** above,as well as against AX30 and AMC30 (**Fig. 2)**. About 21% of the isolated contaminants were resistant to SXT25. Of these, four (36.4%) are *P. aeruginosa* and one (*E. coli)* (26%), as shown in **Table 3**.

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



Antibiotic resistance is largely caused by the usage of antibiotics [25]. According to certain research on AST patterns, there is a correlation between the community's widespread use of antibiotics and irrational antibiotic usage, which could influence an individual's antibiograms [26, 27]. 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. 3**.The incidence of *E. coli* obtained from RTES is slightly lower than what was previously reported (34%) in Pakistan [28] and (8.7%) in Nigeria [21]. *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 [29]. The presence of *E. coli* in RTES samples could suggest poor hygienic practices among the street food vendors, and it is a fecal contamination indicator.

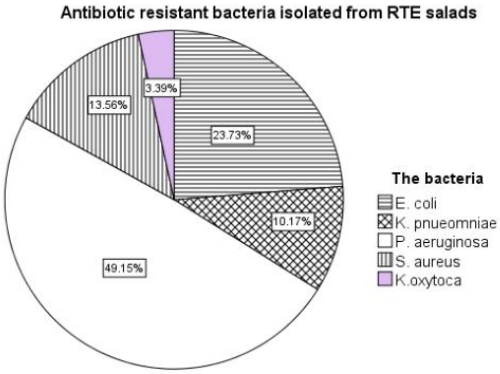


Fig. 3. Proportions of antibiotic resistant bacteria isolated from RTES grouped by species

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 AX30 and AMC30 (Pearson's Chi-square = 299.05; $p< .01$) to which they were resistant to the two antibiotics.

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 [30, 31], where about 1% of deaths annually are attributed to food-related contamination by *S. aureus* [31]. As little as 100 thousand *S. aureus* in food may have detrimental effects. *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 infective decontamination during preparation [32]. Consumption of contaminated RTES with such a pathogen may have serious human health consequences, particularly for children, elders, and immunologically compromised persons [5, 33].

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 [33]. *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 [34]. 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 [35].

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 [36]. Our study shows that *Klebsiella oxytoca* was resistant to AX30 and AMC30 (**Table 3**). 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 [37].

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4. CONCLUSION

The microbial quality of RTES available in the surveyed areas raises health concerns. All tested samples had bacterial loads beyond permissive levels. *Pseudomonasaeruginosa* 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 AX30 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 to ensure RTES are not the cause of food-borne outbreaks in

our community and vehicles of dissemination of antibiotic-resistant pathogens. 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.

CONSENT (WHERE EVER APPLICABLE)

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.
No manuscripts will be peer-reviewed if a statement of patient consent is not presented during submission (wherever applicable).

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REFERENCES

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