Article

ISOLATION AND ANTIMICROBIAL SUSCEPTIBILITY OF BACTERIA FROM FRUIT SMOOTHIE PRODUCED IN ENUGU METROPOLIS

ABSTRACT

Fruit smoothie are blended beverages made primarily from fresh or frozen fruits, often combined with other ingredients. The aim of this study is to isolate and determine the Antimicrobial susceptibility of bacteria present in fruit smoothie.This is a cross-sectional study carried out between August -October 2024. Twenty analyzed fruit smoothie samples were collected from eight(8) vendors in Enugu metropolis , the sample was homogenized and cultured on MaCconkay and chocolate agar with 2UL wire loop.isolates were identified using gram staining, catalase, methyl red, oxidase and coagulase test, it ws subjected to antimicrobial susceptibility using the kirby-Bauer method. Out of the 20samples analyzed, the findings revealed that staphylococcus aureus 6(54.5%) was the most frequently isolated bacterium, followed by pseudomonas aeruginosa 3(27.3%) and Escherichia coli 2(18.2%).In Antimicrobial susceptibility, Levofloxacin (83.3%) and Ciprofloxacin (60%)has the highest sensitivity against staphylococcus aureus while Ampicillin and Clindamycin exhibitedtge highest resistance. Ofloxacin and Ciprofloxacin maintain the highest sensitivity, Nitrofurantoin and Chloramphenicol shows the highest resistance in treating E.coli and Pseudomonas aeruginosa.In Conclusion, These reslts are consistent with previous studies who reported high contamination of ready to eat food products by Staphylococcus aureus due to improper hygiene during preparation.The isolation of E.coli and Pseudomonas aeruginosa aligns with previous studies who identified these bacteria as common contaminants in beverages pointing to issues with water quality and poor handling practices.

Keywords: Smoothie, Antimicrobial susceptibility, pathogens; Staphylococcus aureus, E.coli, Ps. aeruginosa

1.INTRODUCTION

Fruit smoothies, a popular and nutritious beverage may pose significant health risk to consumers due to bacterial contamination. Despite their demonstrated positive effect on various chronic human diseases, fresh fruits and vegetables have recently become the cause of foodborne illness outbreak in developed countries (Skockova et al., 2016)

According to a study conducted by Callejo *et al.* (2015), who summarized reported foodborne outbreaks in the United States (US) and the European Union (EU) between 2004 and 2012, Salmonella was the most prevalent bacterial pathogen (Callejo *et al.,* 2015). The second most common bacterial pathogen was E. coli (Callejo *et al.,* 2015). One of the biggest outbreaks was linked to the consumption of spinach in the US in 2006. Almost 200 people were infected

by the E. coli O157:H7 strain, and three deaths were confirmed. In 2016, a large outbreak of E. coli O157 appeared in the United Kingdom, where the outbreak vehicle was mixed salad leaves (Gobin *et al.,* 2018).

According to previous pathogenic microorganisms. One of the reported outbreaks was smoothie drinks contaminated with hepatitis A in 2016. The Centers for Disease Control and Prevention (CDC) recorded 134 people with hepatitis A after drinking a smoothie containing strawberries imported from Egypt.

Some reports have revealed that antibiotic resistance levels are becoming elevated among food-borne pathogens such as Salmonella and Shigella (Mache, 2015). Although, it is difficult to prove a direct role of drug resistance in bacteria contaminating food items with increased clinical cases of resistant infections, the presence of such bacteria in food items could play a role in the spread of antimicrobial resistance amongst food-borne pathogens (Farzana et al., 2019).

2. MATERIALS AND METHODS

Twenty samples of locally prepared unpasteurized fruit smoothies were collected from eight restaurant in Enugu metropolis from July 2024 to October 2024. Samples were collected in a universal container and transported to the laboratory immediately in an icebox where they were processed.

Pathogenic bacteria such as E.coli, Pseudomonas aeruginosa and Staphylococcus aureus were isolated Samples were confirmed by streaking on MaCconkay and Chocolate AGAR using 2UL wireloop, incubated at 37°C for 24hrs and biochemically tested. In all cases, for the confirmation of the pathogens, typical colonies were identified based on cultural, microscopic and biochemical characteristics (Buchanan et al., 1974). All isolates of pathogenic bacteria were tested for their sensitive to antibiotics by means of the disc diffusion method on Mueller-Hinton AGAR.All disks used in the disk diffusion test are in the following concentrations: Ampicillin (30), Amoxicillin (10), Clindamycin (10), Erythromycin (10), Cetriaxone(30), Gentamicin (10), Ofloxacin (10), Ciprofloxacin(10), Levofloxacin (5), Streptomycin(30) and Chloramphenicol (10). The colonies were picked and spread evenly over the entire Mueller -Hinton AGAR surface.

The antibiotics impregnated discs were then placed onto the surface of the inoculated plates and incubated at 37°C for 16-18hrs. After inhibition, diameters of the zones of inhibition were measured in mmand interpreted as susceptible, intermediate and resistant (CLSL, 2007).

3. RESULTS AND DISCUSSION

Out of the 20 samples analyzed, the prevalence of Staphylococcus aureus, E.coli and Pseudomonas aeruginosa were 6(54.5%), 2(18.2%) and 3(27.3%) respectively (Table1)

Table 1.0: Frequency and Composition of smoothies in different Bars.

BAR	SMOOTHIE CODES	INGREDIENTS	FREQUENCY
A	FA1, FA2, FA3	Banana, coconut, tigernut, date, watermelon	3
B	FB1,	Watermelon, banana, apple, pineapple, milk	1
C	FC1, FC2, FC3	Apple, banana, strawberry, tigernut	3
D	FD1, FD2	Avocado, banana, pineapple, cucumber	2
E	FE1, FE2	Banana, pineapple, watermelon, avocado	2
F	FF1, FF2	Apple, avocado, tigernut, beetroot	2
G	FG1, FG2, FG3	Avocado, banana, tigernut, cucumber, grape	3
H	FH1, FH2, FH3, FH4	Banana, tigernut, watermelon, yoghurt	4

Table 1.0 indicates that Bar H produced the highest number of smoothie samples (4), while Bars A, C, and G each produced three samples.

Table 1.1: Distribution of Isolated Bacteria in fruit smoothies

Bacteria	Smoothie code	Frequency	Percentage(%)
Staphylococcus aureus	FA1, FA2, FA3, FC1, FD1, FF1	6	54.5
Escherichia coli	FG1, FG2	2	18.2
Pseudomonas	FB1, FE1,FE2	3	27.3

aeruginosa			
Total		11	100

From Table 1.1, Staphylococcus aureus was the most frequently isolated organism, representing 54.5% of the total isolates. Pseudomonas aeruginosa followed at 27.3%, while E.coli accounted for 18.3%. This indicates that Staphylococcus aureus may pose a significant contamination risk in fruit smoothies.

These results are consistent with those of Olayemi et al. (2016) and Oyetoyinbo et al. (2020), who reported high contamination of ready to eat food products by staphylococcus aureus due to improper hygiene during preparation. The isolation of E.coli and Pseudomonas aeruginosa aligns with studies by okocha et al. (2019), who identified these bacteria as common Contaminants in beverages, pointing to issues with water quality and poor handling practices.

According to the study conducted in Amravati city, India the incidence of bacterial pathogen recorded was E.coli(40%) followed by pseudomonas aeruginosa (25%) and Staphylococcus aureus (6%) in street vended fruit juice samples (Tambekar et al, 2009). Incident of current study was differing from the study conducted in Asian Countries. The probable reason for difference may be attributed to fruit type, geographical variation and Sanitation habit.

This study also tried to address antimicrobial susceptibility testing by using means of a disc diffusion method on Mueller-Hinton agar. The results of the antibiotic sensitivity test were interpreted amd are presented as the resistant of bacteria isolatesto the antibiotics (Table 2). Most isolates were susceptible to Levofloxacin, Ciprofloxacin, Ofloxacin, Gentamicin and perfloxacin, most of the isolates were resistance to Ampicillin, Amoxicillin and Chloramphenicol.

Table 2 . Antimicrobial susceptibility patterns of pathogenic bacteria isolates from fruit smoothies (in %).

Antibiotics	Bacteria isolates					
	S.aureus N=6		E.coli N=2		Ps.aeruginosa N=3	
	S	R	S	R	S	R
Gentamicin	33.3	66.7	60.0	40.0	60.0	40.0
Amoxicillin	20.0	60.0	50	50	50	20
Ampicillin	16.7	83.3	20	80	20	80
Chloramphenicol	20.0	80.0	40	60	40	60
Erythromycin	33.3	66.7	20	80	20	80

Ofloxacin	50.0	50.0	80.0	20.0	80.0	20.0
Ciprofloxacin	70.0	30.0	80.0	20.0	80.0	20.0
Levofloxacin	83.3	12.7	60.0	40.0	60.0	40.0

The antimicrobial susceptibility tests demonstrated that Staphylococcus aureus exhibited high sensitivity to fluoroquinolones, particularly Levofloxacin (83.3%) and Ciprofloxacin (66.7%). This aligns with findings by Afolabi et al. (2018) and Bello et al. (2021), who highlighted fluoroquinolones as effective treatments for Staphylococcus aureus infections. However, significant resistance to beta-lactans, such as Ampicillin (83.3%) and Clindamycin (83.3%), was observed, supporting reports by Ekundayo et al. (2017) that suggest the increasing prevalence of beta-lactamase production among foodborne pathogens.

For gram- negative bacteria, E.coli and Pseudomonas aeruginosa showed high sensitivity to Ofloxacin and Ciprofloxacin (80% each).These findings corroborate those of Iroha et al.(2019) and EZE et al. (2022), who reported the effectiveness of fluoroquinolones against gram-negative bacteria. However, resistance to Nitrofurantoin (80%) and chloramphenicol (80%) was significant, highlighting the limited therapeutic options available for treating infections caused by these pathogens. The intermediate sensitivity observed for some antibiotics, such as Cetriaxone and Gentamicin, suggests a need for careful consideration when selecting antimicrobial agents for treatment.

CONCLUSION

This study identified Staphylococcus aureus, Pseudomonas aeruginosa and E.coli as the primary bacterial Contaminants in fruit smoothies sold in Enugu metropolis.this findings highlight the need for stringent hygiene practices and routine microbial assessment of smoothie to safeguard public health.

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