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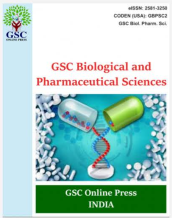

(Research Article)

Detection and profiling of antibiotic resistance among bacteria isolates from new and unused styrofoam and single use plastics sold in Orlu, Imo State Nigeria

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Abstract

Background: Many ready to eat foods are packaged with single use plastics and Styrofoam that are rarely cleaned, washed or sterilized before use by food vendors, at home and public events. These surfaces harbour bacteria which contaminate the food and result in food-borne diseases when consumed along with the food by susceptible individuals, leading to illnesses and possibly death. This study assessed the status of bacterial contamination and antibiotic susceptibility profile of bacteria isolates from new and unused Styrofoam and single use plastics sold in Orlu, Imo State Nigeria.

Methodology: A total of thirty (30) Single Use Plastics bought from random outlets in Orlu international market were analyzed using standard microbiological techniques for bacterial isolation and identification, followed by antibiotic susceptibility testing using the disk diffusion method.

Result: The total bacterial count ranged from 1.1 X 10^5 to 9.0 x 10^5 CFU/ml. Six bacterial genera were identified, with *Staphylococcus aureus* being the most prevalent 70 (32.4%), followed by *Clostridium* sp. 46(21.3%), *Pseudomonas aeruginosa* 37(17.1%), *Bacillus* sp. 35(16.2%), *Streptococcus* sp. 20(9.3%), and *Escherichia coli* 8(3.7%). Antibiotic susceptibility was evaluated against seven commonly used antibiotics: Amikacin, Bacitracin, Ceftazidime, Clindamycin, Gentamicin, Mupirocin, and Ofloxacin. Amikacin demonstrated the highest effectiveness with near-complete susceptibility across most isolates, while variable resistance patterns were observed for other antibiotics. Notable resistance was found against Bacitracin (15.4-50%) and Ofloxacin (8.3-33.3%) across different bacterial species. A Multiple Antibiotic Resistance Index (MARI) analysis revealed high levels of multi-drug resistance, with values ranging from 0.42 to 0.71, being highest in *Clostridium* sp. isolates from both Styrofoam and single-use plastics. All bacterial isolates exhibited MARI values exceeding 0.2, indicating high-risk sources with significant antibiotic exposure.

Conclusion: The presence of these multi-drug-resistant strains on unused food-contact materials raises significant public health concerns, particularly given the widespread use of these materials in food packaging and service industries. These findings emphasize the need for enhanced quality control measures during manufacturing and storage, stricter hygiene protocols, and regular monitoring of antibiotic resistance patterns in food-contact and packaging materials.

Keywords: Styrofoam; Single Use Plastics; Antibiotics Resistance; Food Safety; Nigeria

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1. Introduction

Foodborne disease is caused by bacteria that spread due to inadequate hand washing and insufficient surface disinfection [1]. Despite the possible health hazards, the widespread and uncontrolled use of single-use plastics and Styrofoam to package and serve food without any kind of cleaning or sterilizing has become a part of our everyday life. Despite their convenience, these materials might harbor a variety of bacteria, including potentially harmful pathogens [2]. Food safety and shelf life can be impacted by the spread of infections and spoilage microorganisms due to the adherence and persistence of microbes to surfaces [3]. The potential for antibiotic resistance and these bacteria's capacity to survive on artificial surfaces provide a difficult problem at the nexus of environmental science and public health [4,5].

A major public health concern at the moment is the danger of food poisoning from eating food contaminated with known foodborne pathogens or bacteria resistant to antibiotics [6]. Food-borne illnesses are a serious health concern that can influence socioeconomic advancements in both developed and developing nations and can predispose people to poor health conditions [7]. At least two billion people worldwide suffer from food-borne illnesses each year. These illnesses are seen as one of the biggest public health issues facing the modern world [8]. Food-borne infections are regarded as having severe health hazards in Nigeria, the most populous country in Africa, where they cause about 200,000 fatalities each year [9], along with the associated economic effect and loss of productivity, among other things. Food-borne illnesses are caused by several genera of bacteria. Some, like *Bacillus subtilis* and *Enterobacter cloacae*, cause food to deteriorate [5], while others, like *Clostridium* species, contribute volatile odorous substances produced during the metabolism of microbes, and *Bacillus* species, create mucus and negatively impact human health [2]. *Salmonella*, *Escherichia coli* O157, *Campylobacter*, *Listeria monocytogenes*, *Clostridium perfringens*, *Staphylococcus*, *Shigella*, and *Bacillus* are among the many bacterial pathogens that can harm various foods and subsequently cause illnesses [10], as are *Yesinia enterocolitia*, *Campylobacter jejuni*, and *Campylobacter coli* [11].

Due to the close connection with public health, there is currently a growing interest in food safety and cleanliness as well as the prevalence of food-borne illnesses [8]. According to recent research, these materials may include a variety of microbial communities, including potentially harmful and environmental microorganisms [12, 13]. Due to the significant potential of microbial contamination of foods by various biological hazards, which can result in unnecessary economic burden, preventable deaths, and emotional anguish, microbial food-borne illness is one of the main public health problems [8,14,15]. It has also been suggested that bacteria isolated from surfaces in contact with food might transmit resistance factors when they are subjected to pressure from improper antimicrobial agent application [1].

Like many metropolitan and semi-urban towns in developing nations, the town of Orlu in Imo State, Nigeria, has witnessed a sharp rise in the usage of Styrofoam and single use plastic packaging materials often referred to as 'Takeaway' packs for food packaging by street food vendors, eateries and at public gatherings. Both the personal hygiene of food sellers and the processes of food preparation, storage, transportation, preservation, and distribution expose these street meals and the packaging materials to a multitude of microorganisms [16]. Food poisoning and the spread of antibiotic resistance are at risk due to this trend [16]. The possible significance of Styrofoam and single-use plastics as bacterial transmission vectors and antibiotic resistance reservoirs has received less attention than the well-established environmental effects of plastic pollution. Especially in fast urbanizing towns like Orlu, Nigeria, little is known about the bacterial load on these materials and the antibiotic resistance profiles of the isolates. Our understanding of the possible threats to public health posed by the widespread presence of these elements in the environment is hampered by this knowledge gap.

In order to determine the prevalence of antibiotic-resistant bacteria present in this food packaging material, provide insights into the potential role of these materials as reservoirs for pathogenic bacteria, and inform the public health implications of using these items to package foods without proper handling, this study evaluated the bacterial communities present on Styrofoam and single-use plastics in Orlu and determined their antibiotic resistance profiles.

2. Materials and methods

2.1. Sample Collection

A total of 30 new and unused single use plastics (10 Styrofoam, plastic food packs and toast cups each) were bought from random shops in Orlu main markets Imo state, Nigeria, in August, 2024. These samples were collected while wearing hand gloves, immediately placed inside disinfected plastic bags, and transported to the Microbiology laboratory of Microbiology Department Kingsley Ozumba Mbadiwe University for processing and analysis.

2.2. Isolation and Enumeration

In order to determine the bacterial isolates present, A 10-fold serial dilution was carried out and 100µl of sample from a tube with 10^{-5} dilution was inoculated onto nutrient agar plates using spread plating method, these plates were then incubated at 37 °C for 24 hours. After incubation, visible microbial colonies were counted using viable count method with colony counter, and the CFU/ml was noted [17].

2.3. Bacterial Identification

The bacterial isolates were identified using physical observation of the colony color and shape, followed by Gram's staining, and then biochemical tests which included catalase test, coagulase test, citrate test, indole test, and urease test, which were chosen based on the results of the physical morphological observation and Gram's reaction of the isolates.

2.4. Antibiotic Susceptibility Assay

The bacterial isolates' antibiotic susceptibility was assessed using the procedures outlined by Umar *et al*. [13]. Using a disc diffusion antibiotic susceptibility test, Mueller Hinton Agar (MHA) plates were inoculated with standardized inocula of the identified bacteria using spread plating. Standard discs of Clindamycin, Bacitracin, Ofloxacin, Ceftazidime, Mupirocin, Gentamicin, and Amikacin were then aseptically placed on the inoculated plates using sterile forceps, with the exception of the control dish, which contained only inoculated MHA without antibiotic discs. The setup was then incubated at 35 °C for eighteen hours, zones of inhibition were observed, measured to the nearest millimeter using a meter rule, and interpreted as sensitive or resistant, zone of inhibition of ≤14 is considered as resistant, and ≥19 is considered as susceptible using documented guidelines for antimicrobial susceptibility testing [18].

3. Results

3.1. Isolation and Enumeration

The results of isolation and enumeration (Table 1) revealed that twenty-three (23) samples out of the total of thirty (30) presented visible bacterial growth, and the total bacterial counts isolated from these samples ranged from 1.1 X 10^5 to 9.0 x 10^5 CFU/ml.

Table 1 Bacterial loads of the Styrofoam and Single Use Plastics

	Samples Code	Number of Colonies	Bacterial load (CFU/ml)
Styrofoam	SF1	14	1.4 X 10^5
	SF2	12	1.2 X 10^5
	SF3	5	5.0 X 10^5
	SF4	11	1.1 X 10^5
	SF5	16	1.6 X 10^5
	SF6	12	1.2X 10^5
	SF7	-	-
	SF8	13	1.3 X 10^5
	SF9	-	-
	SF10	-	-
Single Use Plastics	SUP1	6	6.0 X 10^5
	SUP2	12	1.2 X 10^5
	SUP3	-	-
	SUP4	4	4.0 X 10^5
	SUP5	9	9.0 X 10^5
	SUP6	16	1.6 X 10^5

	SUP7	11	1.1×10^5
	SUP8	7	7.0×10^5
	SUP9	8	8.0×10^2
	SUP10	3	3.0×10^5
Single use toast cup	SUTC1	-	-
	SUTC2	5	5.0×10^5
	SUTC3	-	-
	SUTC4	13	1.3×10^5
	SUTC5	5	5.0×10^5
	SUTC6	11	1.1×10^5
	SUTC7	3	3.0×10^5
	SUTC8	7	7.0×10^5
	SUTC9	4	4.0×10^5
	SUTC10	9	9.0×10^5
		216	

Key: - = no visible microbial growth

3.2. Identification of Bacteria

The results of morphological and biochemical identification (Table 2) revealed the presence of *Bacillus* sp., *Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Streptococcus* sp. and *Clostridium* sp in most of the samples. Microorganisms such as *E. coli* and *S. aureus* poses a lot of health hazards to the individuals using these food packaging materials and can result to serious complications when consumes.

Figure 1 below presents the frequency distribution of bacterial isolates in three types of materials: Styrofoam, single-use plastics, and single-use toast cups. The isolates include *Bacillus* sp., *Clostridium* sp., *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Streptococcus* sp. The results indicate variations in bacterial contamination across this food packaging materials, highlighting potential public health concerns in them.

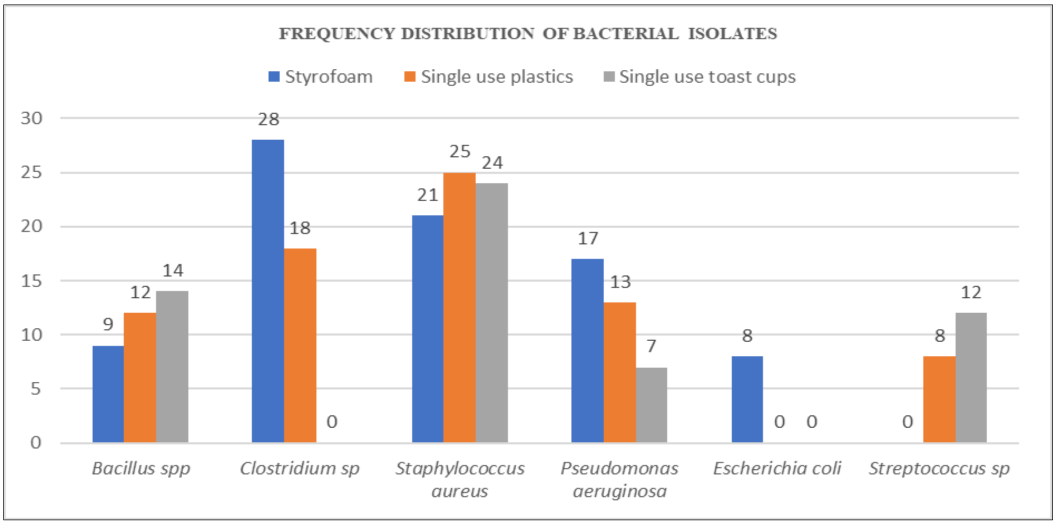

Figure 1 Frequency of Bacterial distribution in Styrofoam, Single use plastics, Single use toast cups

Table 2 Morphological and Biochemical Characteristics of Bacterial species Isolated from Styrofoam and single use plastic samples

Morphological Characteristics				**Microscopic Characteristics**	**Biochemical Test**									**Organism**
Source	Cell Shape	Cell arrangement	Colour	Gram reaction	Motility Test	Catalase Test	Citrate Test	Coagulase Test	Indole Test	Oxidase Test	Methyl red	Nitrate reduction test	VP Test	
Styrofoam and Single use plastics	Rod	Single	Pink	-	+	+	-	-	+	-	+	+	-	*Bacillus* sp
	Cocci	Cluster	Golden-yellow	+	-	+	+	+	-	-	+	+	+	*Staphylococcus aureus*
	Rod	Pairs	Greenish	-	+	+	+	-	-	+	-	+	-	*Pseudomonas* sp
	Cocci	cluster	Milky Yellow	-	-	-	-	-	+	-	-	+	+	*Streptococcus sp*
	Rod	Pair	Pink	-	+	+	-	-	+	-	+	+	-	*Escherichia coli*

Key: + = Positive Reaction; - = Negative Reaction

Table 3 Frequency of occurrence of the bacterial isolates from the surface of the Styrofoam

Bacteria isolates	**Styrofoam**	**Single use plastics**	**Single use toast cups**	**Total**	**Percentage Of Bacterial Occurrence (%)**
Bacillus sp	9	12	14	35	16.2
Clostridium sp	28	18	0	46	21.3
Staphylococcus aureus	21	25	24	70	32.4
Pseudomonas aeruginosa	17	13	7	37	17.1
Escherichia coli	8	0	0	8	3.7
Streptococcus sp	0	8	12	20	9.3
Total	83	76	57	216	100

Figure 2 shows the percentage distribution of bacterial occurrence, providing an overall representation of the prevalence of specific bacterial species across different packaging materials. The bacterial isolates include *Staphylococcus aureus*, *Clostridium* sp., *Pseudomonas aeruginosa*, *Bacillus* sp., *Streptococcus* sp., and *Escherichia coli*.

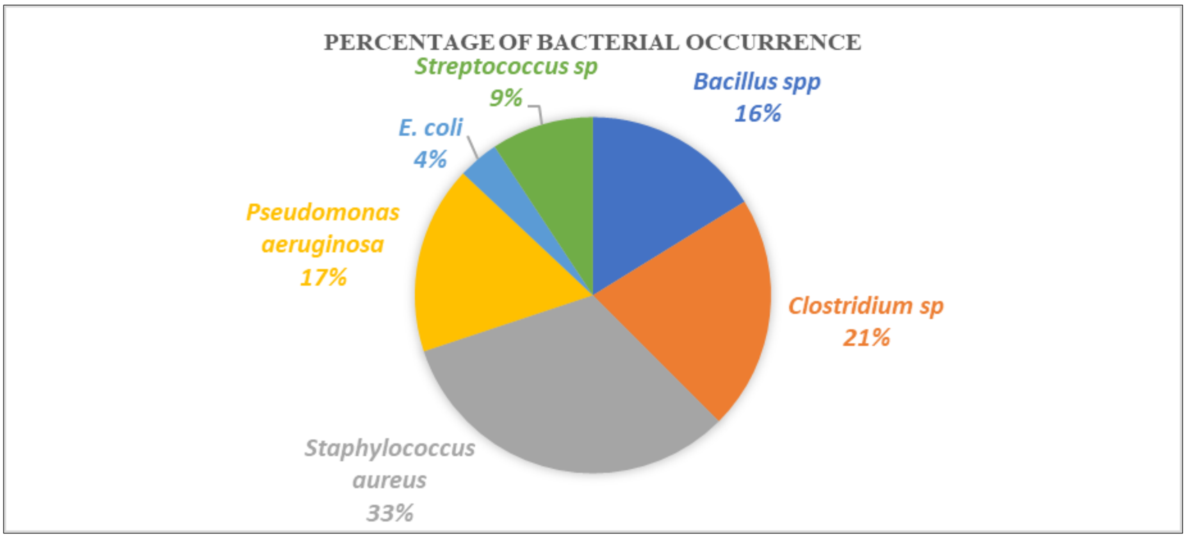

Figure 2 Percentage distribution of bacterial isolates from the surface of Styrofoam, Single use plastics, Single use toast cups

The Table below outlines the antibiotic susceptibility profiles of bacterial isolates from Styrofoam, detailing the number of resistant (R) and susceptible (S) isolates for each antibiotic tested as shown below.

Table 4 Antibiotics susceptibility test of isolates from Styrofoam

Antibiotics	**Conc. (µg)**	***Bacillus* sp.** ***n= 9***		***Clostridium* sp.** **n=28**		***Pseudomonas* sp.** ***n= 17***		***Staphylococcus aureus*** **n=21**		***Escherichia coli*** **n= 8**	
		R (%)	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)
Amikacin	30	0(0.0)	9(100)	0(0.0)	28(100)	0(0.0)	17(100)	0(0.0)	21(100)	1(12.5)	7(87.5)
Bacitracin	10	0(100)	9(100)	4(14.3)	24(85.7)	8(47.0)	9(52.9)	0(0.0)	21(100)	4(50)	4(50)
Ceftazidime	30	1(11.1)	8(88.8)	3(10.7)	25(89.3)	3(17.6)	14(82.4)	2(9.5)	19(90.5)	0(0.0)	8(100)

Clindamycin	15	0(0.0)	9(100)	1(3.6)	27(96.4)	12(70.6)	5(29.4)	4(19.0)	17(81.0)	0(0.0)	8(100)
Gentamicin	30	2(22.2)	7(77.7)	4(14.3)	24(85.7)	9(52.9)	8(47.1)	2(9.5)	19(90.5)	0(0.0)	8(100)
Mupirocin	30	0(0)	9(100)	0(0.0)	28(100)	0(0.0)	17(100)	0(0.0)	21(100)	0(0.0)	8(100)
Ofloxacin	15	3(33.3)	6(66.6)	5(17.9)	23(82.1)	0(0.0)	17(100)	1(4.8)	20(95.2)	2(25)	6(75.0)

KEY: DA= Clindamycin, B = Bacitracin, OFX = Ofloxacin, CAZ = Ceftazidime, MUP = Mupirocin, CN = Gentamicin, AK = Amikacin.

The antibiotic susceptibility profiles of bacterial isolates from single-use toast cups to different antibiotics, providing a comparative analysis of their efficacy.

Table 5 Antibiotics susceptibility test of isolates from single use plastics

Antibiotics	**Conc. (µg)**	***Bacillus* sp *n= 12***		***Clostridium* sp N= 18**		***Pseudomonas* sp *n= 13***		***Staphylococcus aureus* n=25**		***Streptococcus sp* n= 8**	
		R (%)	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)
Amikacin	30	0(0.0)	12(100)	0(0.0)	18(100)	0(0.0)	13(10)	0(0.0)	25(100)	0(0.0)	8(100)
Bacitracin	10	2(16.7)	10(83.3)	4(22.2)	14(77.8)	1(7.7)	12(92.3)	2(8.0)	23(92.0)	3(37.5)	5(62.5)
Ceftazidime	30	2(16.7)	10(83.3)	3(16.7)	15(83.3)	3(23.1)	10(76.9)	0(0.0)	25(100)	0(0.0)	8(100)
Clindamycin	15	0(0.0)	12(100)	2(11.1)	16(88.9)	0(0.0)	13(100)	1(4.0)	24(96.0)	3(37.5)	5(62,5)
Gentamicin	30	0(0.0)	12(100)	0(0.0)	18(100)	0(0.0)	13(100)	2(8.0)	23(92.0)	3(37.5)	5(62.5)
Mupirocin	30	0(0.0)	12(100)	1(5.6)	17(94.4)	1(7.7)	12(92.3)	0(0.0)	25(100)	0(0.0)	8(100)
Ofloxacin	15	1(8.3)	11(91.7)	4(22.2)	14(77.8)	2(15.4)	11(84.6)	3(12.0)	22(88.0)	2(25.0)	6(75)

KEY: DA= Clindamycin, B = Bacitracin, OFX = Ofloxacin, CAZ = Ceftazidime, MUP = Mupirocin, CN = Gentamicin, AK = Amikacin.

Table 6 Antibiotics susceptibility test of isolates from single use toast cups

Antibiotics	**Conc. (µg)**	***Bacillus sp n= 14***		***Pseudomonas* sp *n= 13***		***Staphylococcus aureus* n= 24**		***Streptococcus sp* n= 12**	
		R (%)	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)
Amikacin	30	0(0.0)	14(100)	0(0.0)	13(100)	0(0.0)	24(100)	0(0.0)	12(100)
Bacitracin	10	1(7.1)	13(92.9)	2(15.4)	11(84.6)	0(0.0)	24(100)	4(33.3)	8(66.6)
Ceftazidime	30	1(7.1)	13(92.9)	2(15.4)	11(84.6)	2(8.3)	22(91.7)	0(0.0)	12(100)
Clindamycin	15	0(0.0)	14(100)	0(0.0)	13(100)	4(16.6)	20(83.3)	3(25.0)	9(75.0)
Gentamicin	30	0(0.0)	14(100)	0(0.0)	13(100)	1(4.2)	23(95.8)	1(8.3)	11(91.7)
Mupirocin	30	0(0.0)	14(100)	1(7.7)	12(92.3)	0(0.0)	24(100)	0(0.0)	12(100)
Ofloxacin	15	1(7.1)	13(92.9)	2(15.4)	11(84.6)	4(16.6)	20(83.3)	2(16.7)	10(83.3)

KEY: DA= Clindamycin, B = Bacitracin, OFX = Ofloxacin, CAZ = Ceftazidime, MUP = Mupirocin, CN = Gentamicin, AK = Amikacin.

The Multiple Antibiotic Resistance Index (MARI) analysis revealed varying levels of antibiotic resistance among bacterial isolates from three different sources: Styrofoam, single-use plastics, and single-use toast cups. MARI values ranged from 0.42 to 0.71, indicating significant levels of acquired resistance across all sample sources as shown below.

Table 7 Multiple Antibiotic Resistance Index (MARI) of bacteria isolated from Styrofoam, single use plastics and toast cups

Sample Source	Bacterial Isolate	Antibiotic Code	NAB = 7	MARI Index
Styrofoam	*Bacillus sp*	CAZ-CN-OFX		0.42
	Clostridium sp	B-CAZ-DA-CN-OFX		0.71
	S. aureus	B-CAZ-DA-CN		0.57
	P. aeruginosa	CAZ-DA-CN-OFX		0.57
	E. coli	AK-B-OFX		0.42
Single use plastics	*Bacillus sp*	B-CAZ-OFX		0.42
	Clostridium sp	B-CAZ-DA-OFX-MUP		0.71
	S. aureus	B-DA-CN-OFX		0.57
	P. aeruginosa	B-CAZ-OFX-MUP		0.57
	Streptococcus sp	B-DA-CN-OFX		0.57
Single Use Toast Cup	*Bacillus sp*	B-CAZ-OFX		0.42
	S. aureus	B-CAZ-MUP-OFX		0.57
	P. aeruginosa	CAZ-DA-CN-OFX		0.57
	Streptococcus sp	B-DA-CN-OFX		0.57

4. Discussion

As the need for packaged ready to eat foods continue to grow due to increasing population, restaurant, road side food vendors and event planners have continued to resort to the use of Styrofoam and single use plastics to package and serve foods and drinks. This notwithstanding the cleanliness and/sterility of these packaging materials that are often unwashed before use. This study assessed the status of bacterial contamination and antibiotic susceptibility profile of bacteria isolates from new and unused Styrofoam and single use plastics.

From the study, the surface of new Styrofoam plates and single use plastics plates were contaminated with bacteria belonging to different genera at varying frequencies. The bacteria isolated from the samples, showed a total bacterial count ranging from 1.1 X 105 to 9.0 x 105 CFU/ml (Table 1). The identification of bacteria in each of the samples reveals *Staphylococcus aureus*, and *Clostridium* sp. as the two most frequently occurring contaminants in Styrofoam and single use plastics at 32.4% and 21.3% frequency. These were closely followed by *Pseudomonas aeruginosa* (17.1%) and *Bacillus* sp. (16.2%). *Streptococcus* sp. and *Escherichia coli* were the least bacterial contaminants detected at less significant rates of 9.3% and 3.7% respectively (Table 2, Figure 1). The dominance of *Staphylococcus aureus* across all the samples suggests its resilience and ability to persist under different environmental conditions [2, 3, 5, 19, 20, 21]. As a common pathogen, its presence in food-packaging materials is alarming due to its potential to cause foodborne illnesses, particularly if handling or storage practices are poor. Styrofoam appeared to be the most conducive for *Clostridium* sp. growth, whereas single-use toast cups harbored more *Streptococcus* sp. This suggests variations in microbial adhesion or nutrient availability across materials. Toast cups might provide better conditions for gram-positive cocci like *Streptococcus* sp. *Pseudomonas aeruginosa* and *Bacillus* sp., showed moderate prevalence, contributing 17.1% and 16.2%, respectively. *Pseudomonas aeruginosa* is known for its ability to form biofilms and survive under harsh environmental conditions [22,23], while *Bacillus* sp., may originate from environmental contamination or poor hygienic handling. Contamination of these Styrofoam and single use plastics can come from many possible ways either from lack of quality assurance, poor manufacturing practices, poor storage conditions, coming in contact with contaminated hands, contamination during transportation or even contamination during storage. The presence of these bacterial isolates, identified from Styrofoam and single use plastics, indicates inappropriate storage condition of these products, and poor handling before use. Depending on the consumer health, the isolated organisms can contaminate the food in which these Styrofoam and single use plastics and the drinks which these toast cups are used to serve which might result to food borne diseases and intoxication. The presence and high frequency of these pathogenic bacteria, especially *Staphylococcus aureus* and *Clostridium* sp., in these food-packaging materials confirms the previous reports [13]. This therefore calls for stricter hygiene protocols during manufacturing, storage, and handling.

An antimicrobial evaluation of the bacteria isolated from Styrofoam (Table 4) demonstrated varying susceptibility patterns among bacterial isolates, with Amikacin and Mupirocin being the most effective antibiotics. Resistance was recorded in *Clostridium* sp., *E. coli*, and *Bacillus* sp. to certain antibiotics. The study showed 100% susceptibility to Amikacin (30 µg) across all bacterial isolates, except for *E. coli*, where 12.5% were resistant. This confirms Amikacin's strong efficacy against most of the tested bacteria and highlights its potential as a reliable treatment option [13]. *Clostridium* sp. however, exhibited moderate resistance (14.3%) to Bacitracin, while all other isolates showed high levels of susceptibility, with *Bacillus* sp., *Pseudomonas aeruginosa*, and *Staphylococcus aureus* showing no resistance. This suggests bacitracin's effectiveness, particularly against gram-positive bacteria. *Bacillus* sp. exhibited minor resistance (11.1%) to Ceftazidime (30 µg), while *Staphylococcus aureus* and *Pseudomonas aeruginosa* showed no resistance. Moderate susceptibility was observed for *Clostridium* sp. (10.7%) and *E. coli* (50%), indicating varied efficacy across isolates. Complete (100%) susceptibility to Clindamycin (15 µg) was observed for *Clostridium* sp. and *Bacillus* sp., but *Staphylococcus aureus* showed 19% resistance. Clindamycin appears effective for gram-positive bacteria, though resistance in *Staphylococcus aureus* warrants caution. Resistance to Gentamicin (10 µg) was observed in *Bacillus* sp. (22.2%) and *Clostridium* sp. (14.3%), while all other isolates exhibited 100% susceptibility. Gentamicin remains a strong option for treating gram-negative bacteria like *Pseudomonas aeruginosa* and E. coli. All isolates exhibited 100% susceptibility to Mupirocin (10 µg), confirming it as highly effective against the tested bacteria. Resistance to Ofloxacin (15 µg) was noted in *Bacillus* sp. (33.3%) and *Clostridium* sp. (17.9%), while *Pseudomonas aeruginosa* and *Staphylococcus aureus* showed no resistance. However, *E. coli* showed some resistance (25%). This result corroborates Muriuki *et al*., [24], since Ofloxacin is effective for most bacterial isolates but less so for spore-forming bacteria like *Bacillus* sp. Resistance observed for *E. coli* against Amikacin and for *Bacillus* sp. against Ceftazidime and Ofloxacin highlights the potential for antibiotic resistance to develop, even for generally effective antibiotics.

The antibiotic susceptibility profile of bacterial isolates from single-use plastics (Table 5) revealed varied resistance profiles across the five bacterial genera with Amikacin (30 µg) demonstrating exceptional efficacy, with 100% susceptibility across all bacterial species tested. This consistent effectiveness suggests Amikacin as a reliable broad-spectrum antibiotic against contaminants found on single-use plastics. *Bacillus* sp. showed limited resistance, with 16.7% resistance to both Bacitracin and Ceftazidime, and 8.3% to Ofloxacin as was also found in [25]. Complete susceptibility was maintained to Amikacin, Clindamycin, Gentamicin, and Mupirocin. *Clostridium* sp. however exhibited the highest resistance rates among all species, particularly to Bacitracin and Ofloxacin (22.2% each), followed by Ceftazidime (16.7%). It however, showed complete susceptibility to Amikacin and Gentamicin. *Pseudomonas* sp. demonstrated moderate resistance levels, with highest resistance to Ceftazidime (23.1%) and Ofloxacin (15.4%). Notably, it maintained complete susceptibility to Amikacin, Clindamycin, and Gentamicin. *Staphylococcus aureus* showed varied resistance patterns, with highest resistance to Ofloxacin (12.0%) and lower resistance rates to Bacitracin, Gentamicin, and Clindamycin at 8.0%, 8.0%, and 4.0% respectively. *Streptococcus* sp. exhibited higher resistance levels to multiple antibiotics, with 37.5% resistance to Bacitracin, Clindamycin, and Gentamicin. However, it maintained complete susceptibility to Amikacin, Ceftazidime, and Mupirocin. Comparatively, Gram-positive organisms (*Staphylococcus*, *Streptococcus*) generally showed higher resistance rates compared to Gram-negative bacteria (*Pseudomonas*), except for *Clostridium* sp. These findings highlight significant concerns regarding the presence of antibiotic-resistant bacteria on single-use plastics, particularly given their unregulated and widespread use in food packaging by road side food vendors, eateries and public event planners. The varied resistance patterns observed emphasize the importance of proper storage, and handling to minimize bacterial contamination and potential spread of resistant strains.

The antibiotics profile of bacteria isolates from single use toast cups (Table 6) presents the percentage of isolates resistant (R) and susceptible (S) to different antibiotics, providing a comparative analysis of their efficacy. The study showed complete 100% susceptibility of all the isolates, to Amikacin (30 µg), except for *Streptococcus* sp., where 16.7% were resistant. This indicates amikacin's strong efficacy and reliability as a broad-spectrum antibiotic. High resistance to Bacitracin (10 µg) was observed in *Streptococcus* sp. (33.3%) and moderate resistance in *Pseudomonas aeruginosa* (15.4%). However, *Bacillus* sp. and *Staphylococcus aureus* showed low resistance at 7.1% and 4.2%, respectively, suggesting it is more effective for gram-positive bacteria than for *Pseudomonas aeruginosa*. Resistance was however noted in *Streptococcus* sp. (25%) and *Pseudomonas aeruginosa* (15.4%), to Ceftazidime (30 µg), while susceptibility in *Staphylococcus aureus* and *Bacillus* sp. was high, with minimal resistance at 4.2% and 7.1%, respectively. Its variable efficacy highlights its limited use for certain bacteria. Excellent activity (100% susceptibility) of Clindamycin (15 µg) was observed against *Pseudomonas aeruginosa* and *Bacillus* sp. However, *Staphylococcus aureus* showed moderate resistance (16.7%), and *Streptococcus* sp. had notable resistance (25%), suggesting the need for selective use depending on the bacterial isolate. No resistance to Gentamicin (10 µg) was observed for *Bacillus* sp., *Pseudomonas aeruginosa*, or

Staphylococcus aureus. However, *Streptococcus* sp. exhibited significant resistance (8.3%), indicating gentamicin's strong efficacy, particularly for Gram-negative bacteria like *Pseudomonas aeruginosa*. All isolates demonstrated 100% susceptibility to Mupirocin, confirming its effectiveness and potential as a first-line treatment option. Moderate resistance was however recorded in *Bacillus* sp. (7.1%), *Staphylococcus aureus* (16.6%), and *Pseudomonas aeruginosa* (15.4%) to Ofloxacin (15 μg). Despite this, *Streptococcus* sp. exhibited the highest resistance (33.3%), suggesting a decreasing effectiveness against gram-positive bacteria [6]. These demonstrates that Amikacin and Mupirocin are the most effective antibiotics, showing 100% susceptibility across nearly all isolates. Resistance to Bacitracin, Ceftazidime, and Ofloxacin, especially in *Streptococcus* sp., and *Pseudomonas aeruginosa*, highlights the importance of prudent antibiotic use and routine resistance monitoring. While Clindamycin and Ceftazidime remain effective for most isolates, resistance patterns in *Streptococcus* sp. highlight the importance of judicious use.

The Multiple Antibiotic Resistance Index (MARI) analysis (Table 7), revealed varying levels of antibiotic resistance among bacterial isolates from three different sources: Styrofoam, single-use plastics, and single-use toast cups. MARI values ranged from 0.42 to 0.71, indicating significant levels of acquired resistance across all sample sources. *Clostridium* sp. exhibited the highest MARI value (0.71), showing resistance to multiple antibiotics (B-CAZ-DA-CN-OFX). *Bacillus* sp., *S. aureus*, and *Pseudomonas aeruginosa* showed moderate resistance (MARI = 0.42-0.57). *E. coli* demonstrated a MARI value of 0.42, indicating resistance to AK-B-OFX for Styrofoam isolates. In the single use plastics, *Clostridium* sp. maintained the highest MARI value (0.71), showing resistance to B-CAZ-DA-OFX-MUP. This was closely followed by *S. aureus*, *P. aeruginosa*, and *Streptococcus* sp., which both exhibited consistent MARI values of 0.57 while *Bacillus* sp. showed a lower MARI value of 0.42. conversely, all isolated bacteria from the single use toast cups (*Bacillus* sp., *P. aeruginosa*, and *Streptococcus* sp.) showed uniform MARI values of 0.57. *Clostridium* sp. consistently demonstrated the highest level of multiple antibiotic resistance (MARI = 0.71) across different sources. A Multi antibiotic resistance index (MARI) value >0.2 indicates high-risk contamination sources, and all isolates exceeded this limit. The consistency of MARI values (0.57) across different species in toast cups suggests possible cross-resistance development. MARI values >0.4 indicate isolates originating from high-risk sources with high antibiotic exposure. The elevated MARI values across all sources (0.42-0.71) suggest significant antibiotic selection pressure in these environments. The presence of multi-drug resistant strains on food packaging materials and contact surfaces poses potential public health risks [26]. The high MARI values observed across all three sources indicates significant antibiotic selection pressure in the environment, Potential for horizontal gene transfer among bacterial populations, Need for enhanced sanitation protocols and antibiotic stewardship [1].

In Nigeria, Styrofoam and single use plastics have been widely used without considering if they sterile or not. This study has shown that new and unused Styrofoam and single use plastic sold in Orlu Orlu, Imo State Nigeria are largely contaminated with various Bacteria that are resistant to commonly used antibiotics. This poses significant health risks to consumers. The findings of these study underscore the importance of maintaining cleanliness in food service settings and of packaging materials to mitigate the risks of foodborne illnesses associated with contaminated food contact surfaces.

5. Conclusion

This study shows that Styrofoam and single use plastics sold in Orlu are contaminated with resistant bacteria which can cause food borne illness if transferred to food. In order to reduce the bioburden of pathogenic bacterial on single use plastics and Styrofoam used for food packaging to the lowest level, wholesalers as well as retailers should ensure good storage practices. The surface of these packaging materials should also be cleaned or washed before use. This can help reduce the bioburden and prevent the ingestion of resistant pathogenic organisms that can cause food-borne infections. Like the Lagos state government recently outlawed the use of Styrofoam, there is need to regulate the use of these plastic packaging materials due to their potential to spread resistant pathogenic microorganisms, and environmental hazards among other reasons. Awareness must also be raised about the misuse of antibiotics in animal husbandry.

Compliance with ethical standards

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Disclosure of conflict of interest

We declare no conflict of interest.

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