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Antibiotic Resistance Patterns of Bacterial Isolates From Ready to Eat Jollof Rice Sold in Different Restaurant in Abakaliki and Ikwo, Ebonyi State, Nigeria

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ABSTRACT

The emergence of antibiotic resistance among contaminated pathogens represents a considerable public health dilemma, particularly in the context of ready-to-eat foods. The goal of this study was to look at the antibiotic resistance patterns of bacteria found in ready-to-eat jollof rice from different eateries in Abakaliki and Ikwo, Ebonyi State. Ten samples of jollof rice were randomly taken from different restaurants and tested for bacteria using established procedures. The standardised Kirby Baur disc-diffusion method was used to find out how susceptible the isolates were to 12 popular antibiotics. The results showed that the viable counts were higher than the limit set by the Centres for Disease Control, ranging from 2.0 x 10^4 cfu/g to 1.2 x 10^6 cfu/g. Some samples had coliform bacteria in them, with counts as high as 4.4 x 10^2 cfu/g. This shows that the samples were very contaminated and that food was not handled properly. Eight harmful bacteria were isolated, including *Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, Shigella sp*, *Pseudomonas aeruginosa*, *Micrococcus sp, and Bacillus sp*. The antibiotic susceptibility testing showed very high rates of resistance, especially to Ampicillin (80%) and Erythromycin (75%). Chloramphenicol, on the other hand, showed the highest susceptibility (85%). The results indicate a concerning trend of antibiotic resistance associated with the inappropriate use of antibiotics in food production. The study also shows how important it is to enhance food safety measures, train vendors, and raise public health awareness. To protect public health, there needs to be more regulatory control and cooperation among health authorities to lower the hazards that come with microbial contamination and antibiotic-resistant bacteria.

Keywords: Antibiotic resistance, food safety, public health, susceptibility, foodborne pathogens.

Introduction

Rice is the seed of the monocot genus Oryza, belonging to the grass family Poaceae (formerly Graminae), which comprises twenty wild species and two cultivated varieties: *Oryza sativa* (Asian rice) and *Oryza glaberrima* (Ajala and Gana, 2015). Rice is cultivated in all ecological dietary zones and tropical areas of Nigeria (Oluwafemi and Simisaye, 2005). The two prevalent rice types planted in Nigeria are *Oryza sativa* and *Oryza glaberrima* (Ajala and Gana, 2015), primarily grown in Abakiliki, South-East Nigeria (African rice). Rice is a crucial cereal in human nutrition, consumed by around 75% of the global population, and it holds similar significance with wheat as a primary food source for humanity. Rice is regarded as the premier staple food among all grains for more than 3 billion individuals, representing over half of the global

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population (Oluwafemi and Simisaye, 2005). Rice comprises minerals including calcium, magnesium, and phosphorus, as well as trace amounts of iron, copper, zinc, and manganese (Davidson, 2014). In certain instances, plants gathered as raw food are transported to an open market for public sale. The purchased food goods are subsequently brought home and transformed into various delicacies as desired (Christison *et al.,* 2008). These culinary items purchased from the market are prepared for consumption both at home and in restaurants. Energy is essential for the numerous enzymatic activities necessary for muscle movement, digestion, respiration, excretion, and reproduction (Sanni *et al.,* 2005). The pathogenic contamination of processed, ready-to-eat jollof rice offered in restaurants is a significant problem in the Abakaliki and AE-FUNAI University communities (Mohammed & Shehasen, 2020). Contaminated food from these restaurants presents several health hazards (Henry *et al.,* 2017). The contamination of ready-to-eat jollof rice from sold foods by *Escherichia coli, Klebsiella spp., Proteus spp., Salmonella spp., Pseudomonas spp., and Enterobacter spp*. is a significant concern due to the potential for these organisms to induce outbreaks of foodborne illnesses such as gastroenteritis, dysentery, and typhoid fever (Oluyege *et al.,* 2009). No documented evidence exists regarding microorganisms linked to jollof rice sold near Alex-Ekwueme Federal University, Ndufu-Alike, Ikwo, Ebonyi State. This study was conducted to examine the pathogenic organisms present in jollof rice sold at several eateries in and around the Alex-Ekwueme Federal University, Ndufu-Alike, Ikwo campus. Ready-to-eat items available in food service centres are conveniently consumed by a varied demographic, including students, employees, and other personnel. Numerous lines of evidence suggest that food sold in restaurants may get contaminated with microorganisms. Claims have emerged that Jollof rice served in restaurants is linked to reported health issues among consumers following its consumption, and research indicates that small and medium-sized food service establishments frequently play a significant role in the transmission of foodborne illnesses (Ajao & Atere, 2009; Chijioke *et al*., 2023). Research investigations have unequivocally demonstrated that biological pollutants are the primary cause of foodborne illnesses. In the developing world, including Nigeria, health risks arise from the initial contamination of raw foods with pathogenic bacteria, followed by contamination from food handlers during preparation due to cross-contamination, the survival of pathogens during preparation, microbial proliferation post-cooking, and during the holding of cooked foods (Mohammed & Shehasen, 2020). Therefore, it is essential to examine the Jollof rice offered in prominent cafés and restaurants in Abakaliki for contamination by germs linked to foodborne illnesses. The project will benefit residents by enhancing consumer understanding on food safety, prompting informed decisions about dining options, and guiding actions to mitigate disease. The study will assist food vendors in mitigating the presence of pathogenic bacteria such as Staphylococcus aureus and Salmonella typhi in Jollof rice, given their critical significance to human health. Therefore, it is imperative to investigate these pathogens due to their association with food poisoning and to prevent their proliferation. Ultimately, the findings will assist restaurant proprietors in enhancing their food preparation and handling procedures, resulting in superior quality cuisine. The study will aid health workers in accurately diagnosing health issues among residents and consequently, disclose the hygienic standards practiced by food handlers and suppliers during service delivery and enhancements.

Material and Method

Sample collection

A total of 10 samples were purchased for the purpose of the study. About 100g of Jollof rice Samples were collected from five distinct restaurants, with each location—either within Abakaliki or Ikwo local government—being sampled in triplicate. All samples were collected in restaurant serving plates that were securely covered, labelled, and transported on ice to inhibit bacterial growth during transit

to the Microbiology Laboratory, Department of Microbiology, Alex Ekwueme Federal University Ndufu-Alike, Ikwo, Ebonyi State. They were subsequently stored at 4°C until analysis was conducted.

Sample Preparation

To homogenise each food sample, ten (10) grammes were blended in 100 millilitres of regular saline (1 gramme in 10 millilitres). This was designated as a 1:10 dilution, which also serves as the stock or homogenate. The homogenate was subsequently serially diluted to 1:105 by transferring 1 ml to a test tube containing 9 ml of sterile normal saline (1:102). The process was subsequently continued in a serial manner up to (1:105).

Determination of Total Bacterial Load/Total Heterotrophic Bacterial Load

About 1ml of dilution 10^3, 10^4 and 10^5 tubes was seeded in nutrient agar using pour plate method. This was followed by incubation at 37°C for 24 hours under aerobic atmosphere condition for total aerobic plate count as described by Lambu *et al.,* (2022). Growth observed were identified and counted or enumerated as colony forming unit per gram.

Sterilized platinum inoculation loops (Thomas Scientific, USA) were used to pick distinct colony of isolated bacteria randomly from mixed culture after counting and streaked on Nutrient Agar (NA) to obtain pure bacterial sub-cultures, after which they were incubated at 37°C for 24 hours. The stock culture were later inoculated on agar slant and stored at 4° C.

Characterization of Bacteria Isolated from Jollof Rice samples: Pure culture of isolate were obtained by repeated subculture of distinct colony on some selective media (i.e MacConkey, CLED, blood agar and SSA agar) and characterized based on colonial morphology, microscopy (Gram staining), and biochemical tests as described by Cheesbough, (2006).

Antibiotic Susceptibility Testing Ampicillin (AS) 10 µg, Tetracycline (TE) 30 µg, Streptomyc (S) 10 µg, Ciprofloxacin (CP) 5 µg, Ofloxacin (OF) 5 µg, Roxithromycin (RO) 15 µg, Levofloxacin (LE) 5 µg, Cloxacillin (CX) 1 µg, Chloramphenicol (C) 30 µg, Gentamycin (GN) 10 µg. Thus, the test was done to Figure out how effective the antibiotics can control isolates from the samples. The standardized inoculum of isolates were spread evenly on a freshly prepared Muellar Hilton Agar (Hi-Media, India). The inoculums were allowed to dry for 5 minutes.

The bacteria were screened for their susceptibility to medical preparations of Antibiotics Used: Co-trimoxazole (BA) 25 µg, Erythromycin (E) 15 µg, with lid in place. The antibiotic discs were seeded on the inoculated plates under sterile condition. The cultures were then incubated at 37°C for 24 hours after allowing the disc to diffuse within for some times. The plates were examined for zones of inhibition (Prescott *et al*., 2008; Barth *et al.,* 2009).

After 24 hours of incubation, each plate was examined and the diameters of the zones of inhibition were measured. The measurements was compared to the standard reference chart provided by CLSI (Clinical and Laboratory Standards Institute) to determine whether the bacteria are: Susceptible (S)**:** Clear zone is large; the bacteria are sensitive to the antibiotic. Intermediate **(I):** Clear zone is moderately sized; the bacteria have partial resistance. Resistant (R): Clear zone is small or absent; the bacteria are resistant to the antibiotic.

The multiple antibiotic resistant indexes (MARI) for each isolates were equally derived using the mathematical expression of which is given as:

MAR index = a/b

Where **a**- represent the number of antibiotics to which the isolates was resistant and

b- The total number of antibiotics against which an individual isolate was tested (Chijioke *et al*., 2023).

Results and Discussion

The total Aerobic Bacteria and Total Coliform Count of Jollof Rice Samples Collected from Restaurant in Abakaliki and Ikwo indicates the total aerobic bacteria counts ranging from 4.1 X 104 Cfu/ml (highest count) to 2.1 X 103 Cfu/ml (the lowest). While the total coliform count ranged from 3.5 X 100 (highest count) to 0 (Lowest Count) in Table 1.

The Table 2 summarizes the prevalence of various bacterial species across ten different Jolllof rice samples, in which results indicate that Staphylococcus aureus is the most prevalent, found in six of the ten samples (30%), suggesting a significant presence in the sample. *E. coli* and *Shigella spp.* show lower

Table 1: Total Aerobic Bacteria and Total Coliform Count of Jollof Rice Samples Collected from Restaurant in Abakaliki and Ikwo.

S/NO.	Aerobic bacteria count (cfu/ml) on NA	Total coliform count (cfu/ml) on NA
A1	2.1×10^3	0
A2	3.0×10^3	0
A3	2.2×10^4	2.0
A4	8.3×10^4	3.5
A5	4.1×10^4	2
I1	6.9×10^4	0
I2	3.9×10^4	0
I3	5.0×10^3	7.2×10^1
I4	4.6×10^4	4.4×10^2
I5	9.3×10^5	4.2×10^2
NAFDAC/WHO STANDARD	**(1×10^2 cfu/ml)**	**0 (ZERO)**

KEY: **NA** = Nutrient Agar, **A** = Abakiliki **I** = Ikwo

Table 2: Frequency of Occurrence of Bacterial species in the food samples

	SAMPLES OF JOLLOF RICE											
Bacterial Species	**A1**	**A2**	**A3**	**A4**	**A5**	**I1**	**I2**	**I3**	**I4**	**I5**	**Total**	**Percentage (%)**
Staphylococcus aureus	+	+	-	-	+		+	+	-	+	6	30%
E. coli	-	-		+	-	-	+		+	-	2	10%
Shigella spp	-	-	+	-	-	-	-	-	-	-	1	5%
K. pneumoniae	-	-	-	+		+			+		3	15%
P. aeruginosa	-	-	-	-	+	-	-	+	-	-	3	15%
Proteus sp	-	-	-	-	-	+	-	-	-	-	1	5%
Micrococcus sp	-	+	-	-	-	-	-	-	-		1	5%
Bacillus sp	+	-	+	-	-	-	-	-	-	+	3	15%

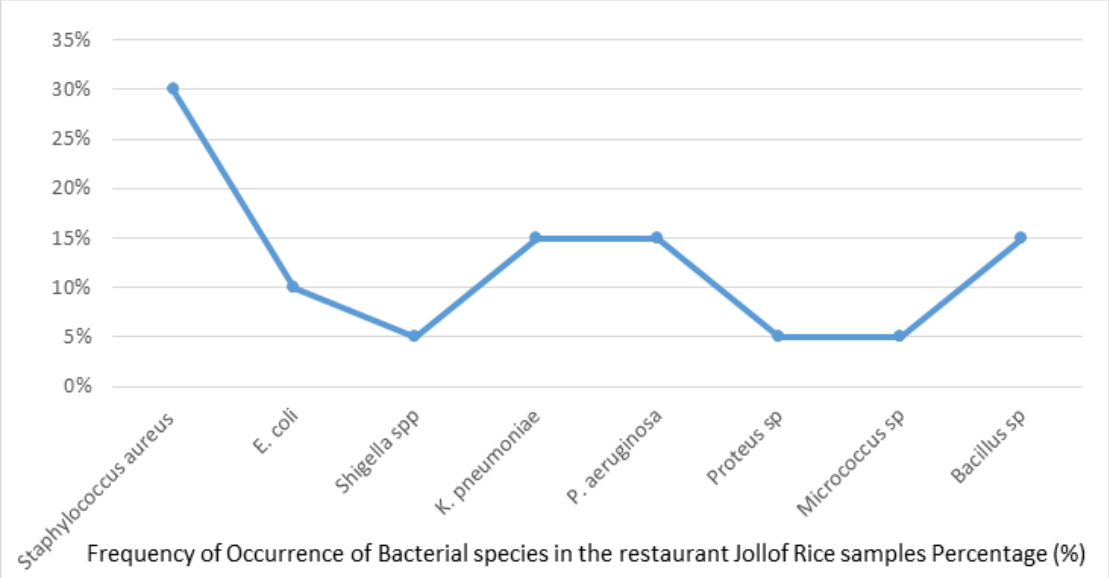

Figure 1: Percentage Frequency of occurrence of various Bacteria species

Table 3: Anti-biogram profile of Twenty Bacteria Isolates from Jollof Rice

	Antibiotic Tested											
	Breaking point {Zones of inhibition (mm)}											
Isolates	**BA**	**E**	**AS**	**TE**	**S**	**CP**	**OF**	**RO**	**LE**	**CX**	**C**	**GN**
CR-A	R	S	S	I	S	S	I	R	S	I	S	S
CR-B	S	R	R	R	I	S	S	R	S	R	S	S
KL-A	R	I	I	R	S	I	R	R	S	R	S	S
KL-B	I	R	S	I	R	R	S	S	R	S	R	S
TA-A	R	R	R	R	S	S	S	R	R	R	S	S
TA-B	R	R	R	I	R	S	R	R	S	R	S	S
CH-A	R	R	R	R	S	R	S	R	S	R	S	R
CH-B	S	R	R	R	R	S	I	R	R	R	S	R
CK-A	R	S	R	S	I	S	S	R	S	I	S	S
CK-B	R	R	R	R	S	R	R	R	S	R	S	R
MU-A	R	R	R	I	R	S	S	R	R	R	S	S
MU-B	R	R	R	S	S	S	R	R	R	S	S	S
DK-A	R	S	R	R	R	S	S	R	S	R	S	S
DK-B	R	R	S	S	S	R	R	S	S	R	S	S
TC-A	R	R	R	R	R	S	R	R	S	R	S	S
TC-B	R	S	R	R	S	S	S	R	S	R	S	S
BK-A	R	R	R	R	R	R	R	R	S	R	S	R
BK-**B**	S	R	R	R	S	R	S	R	S	R	R	S
K.K-A	S	R	R	S	I	S	R	R	S	S	I	S
K.K-B	R	R	R	R	S	I	S	R	S	S	S	R

Key: R: resistance, I: intermediate, S: susceptible.
Antibiotics Used Co-trimoxazole (BA) 25 µg, Erythromycin (E) 15 µg, Ampicillin (AS) 10 µg, Tetracycline (TE) 30 µg, Streptomyc (S) 10 µg, Ciprofloxacin (CP) 5 µg, Ofloxacin (OF) 5 µg, Roxithromycin (RO) 15 µg, Levofloxacin (LE) 5 µg, Cloxacillin (CX) 1 µg, Chloramphenicol (C) 30 µg, Gentamycin (GN) 10 µg.

Table 4: Summary of Each Isolate Reaction to all the Antibiotic Tested measured by Zone of Inhibition and Determine by CLSI breaking Point Guideline.

S/N	Isolates	Resistant n (%)	Intermediate n (%)	Sensitive N (%)
1	CR-A	6 (50.00)	1 (8.33)	5 (41.67)
2	CR-B	8 (66.67)	1 (8.33)	3 (25.00)
3	KL-A	5 (41.67)	2 (16.67)	5 (41.67)
4	KL-B	6 (50.00)	1 (8.33)	5 (41.67)
5	TA-A	8 (66.67)	0 (0.00)	4 (33.33)
6	TA-B	7 (58.33)	1 (8.33)	4 (33.33)
7	CH-A	9 (75.00)	0 (0.00)	3 (25.00)
8	CH-B	6 (50.00)	1 (8.33)	5 (41.67)
9	CK-A	5 (41.67)	1 (8.33)	6 (50.00)
10	CK-B	8 (66.67)	0 (0.00)	4 (33.33)
11	MU-A	6 (50.00)	1 (8.33)	5 (41.67)
12	MU-B	6 (50.00)	1 (8.33)	5 (41.67)
13	DK-A	7 (58.33)	0 (0.00)	5 (41.67)
14	DK-B	6 (50.00)	0 (0.00)	6 (50.00)
15	TC-A	8 (66.67)	0 (0.00)	4 (33.33)
16	TC-B	7 (58.33)	0 (0.00)	5 (41.67)
17	BK-A	8 (66.67)	0 (0.00)	4 (33.33)
18	BK-B	6 (50.00)	0 (0.00)	6 (50.00)
19	K.K-A	6 (50.00)	1 (8.33)	5 (41.67)
20	K.K-B	8 (66.67)	0 (0.00)	4 (33.33)

prevalence, with only two (10%) and one (5%) occurrence respectively, while *K. pneumoniae* and *P. aeruginosa* each account for three occurrences (15%). *Proteus sp, Micrococcus sp, and Bacillus sp* are less common, with each representing only 5% to 15% of the total isolates.

The Table 3 outlines the antibiotic resistance patterns of various bacterial isolates across multiple antibiotics, including Co-trimoxazole (BA), Erythromycin (E), Ampicillin (AS), Tetracycline (TE), Streptomycin (S), Ciprofloxacin (CP), Ofloxacin (OF), Roxithromycin (RO), Levofloxacin (LE), Cloxacillin (CX), Chloramphenicol (C), and Gentamicin (GN). Each isolate is categorized as resistant (R), intermediate (I), or susceptible (S) to these antibiotics. For example, isolate CH-A shows resistance to all tested antibiotics except for S and I, indicating a high level of multi-drug resistance, while isolate TA-B has a mix of resistance and intermediate susceptibility. Overall, many isolates exhibit significant resistance, particularly to E, AS, and R antibiotics.

The antibiotic susceptibility profile of various isolates, detailing the number and percentage of resistant, intermediate, and sensitive to a total of 12 antibiotics (Table 4). Each row corresponds to an isolate, with noTable resistance levels observed across the samples. Isolate CH-A shows the highest resistance at 75%, while isolates like TA-A and TA-B also exhibit significant resistance (66.67%). Conversely, several isolates maintain a reasonable percentage of sensitivity, with some, such as DK-B and CK-A, having 50% sensitivity rates.

The result of high viable heterotrophic bacteria counts of the rice samples, may be suggestive of exposure

to atmosphere, where the environmental condition may be questionable. Also, the high counts may be attributed to carelessness of the vendor. Similar viable counts have been observed by Olaoye *et al.,* (2010), where they obtained a bacteria count ranged from 2.0 x 10^4 cfu/g to 1.2 x 10^6 cfu/g for bacteria. In a similar report, viable heterotrophic bacterial counts ranged from 3.0 x 10^3 to 7.0 x 10^4 cfu/g. The heterotrophic bacteria count in these foods clearly, is higher than Centre of Disease Control (CDC) (2006) set standard of 10^2. Relatively high temperature in Nigeria promotes rapid growth of pathogenic bacteria and poor method of food preservation technique, as such could encourage elevated heterotrophic count in their food (Chijioke *et al.,* 2023).

The present study revealed the presence of coliform in some rice samples. The count ranged from zero to 4.4 x 10^2 cfu/g. This indicate contamination of some restaurant food with coliform bacteria which is a total deviation from CDC food safety value of Zero in food substance. The coliform presence can be linked to poor processing of food and washing of cooking utensils like plates, knife and contaminated water, this finding agrees with Lambu *et al.,* (2022) and Musa *et al.,* (2022) as they recorded coliform contamination of jollof rice Served in Plateau State University Community for Pathogenic Organisms.

High rates of resistance to a number of antibiotics are revealed by the susceptibility profile of bacteria isolated from ready-to-eat jollof rice in Abakaliki and Ikwo. Ampicillin exhibits the highest resistance at 80%, followed by co-trimetazole and erythromycin at 75%. Chloramphenicol exhibited the highest susceptibility rate at 85%, suggesting it may be a more successful treatment option for these isolates. The frequency of resistance is attribuTable to the excessive and improper use of antibiotics in food production and processing, alongside insufficient food safety standards (Ventola, 2015). Research conducted by Hossain *et al*., (2010) and Dallenne *et al*., (2010) substantiates these results, revealing analogous resistance patterns in foodborne pathogens and highlighting a worldwide issue of antibiotic resistance in food products. The anti-biogram profiling results of the bacterial isolates reveal considerable variability in antibiotic resistance, with MARI values between 0.25 and 0.83, indicating significant public health implications. Factors contributing to this resistance levels encompass antibiotic misuse, inadequate cleanliness in food processing, environmental exposure to antibiotics, and the clonal dissemination of resistant isolates.

The results underscore the pressing necessity for enhanced oversight of antibiotics and food safety protocols to reduce the threat of antibiotic-resistant diseases. The current data indicate various commonalities and trends consistent with earlier investigations. Dallenne *et al*., (2010) conducted a study that revealed comparable levels of resistance in food goods, highlighting a troubling occurrence of multi-drug-resistant microorganisms. Hossain *et al*., (2010) identified substantial antibiotic resistance in ready-to-eat meals, underscoring the potential for these food sources to include hazardous microorganisms resistant to prevalent antibiotics. Furthermore, the elevated MARI values identified in this investigation, especially in isolates such as BK-A, correspond with previous research indicating rising resistance levels attributed to antibiotic usage and insufficient food safety measures (Ventola, 2015). The uniformity observed in studies highlights the enduring issue of antibiotic resistance in foodborne pathogens, requiring continuous monitoring and intervention measures to safeguard public health.

Conclusion

This study underscores significant issues pertaining to the microbiological safety of ready-to-eat jollof rice marketed in Abakaliki and Ikwo, indicating elevated viable heterotrophic bacteria levels and the detection of coliform bacteria, which contravene existing safety criteria. Poor vendor hygiene, bad food preservation procedures, and environmental factors all play a role in these contaminations, which together make food less safe. The results are in line with other research done in Nigeria, showing that microbial contamination

in street foods is a big problem that affects public health. The antibiotic susceptibility testing also showed worrying resistance patterns, especially against Ampicillin and Erythromycin, which are two antibiotics that are often used. It also showed that Chloramphenicol was a better therapeutic option. This resistance can be traced back to things like not using antibiotics correctly and not having enough safety precautions in place for food. The differences in the Multiple Antibiotic Resistance Index (MARI) between isolates show that we need to do a better job of managing antibiotics and making sure that food is safe. The report recommends increased awareness and education for both food vendors and customers, as well as stricter oversight of food hygiene procedures, to reduce the dangers posed by antibiotic-resistant organisms and protect public health.

Recommendation

To improve food safety and lower the risk of contamination by bacteria in ready-to-eat jollof rice, food vendors need to be taught how to handle food safely and follow good hygiene procedures. Food places should be checked on a regular basis to make sure they follow safety rules, and microbiological tests should be done to find contamination early. Public health initiatives can teach people how to find safe places to get food and how important it is to keep food clean. Also, sellers should learn better ways to keep food fresh so that bacteria can't thrive. To fight antibiotic resistance, it's important to encourage ethical antibiotic usage in food production. Ongoing study and monitoring of resistance patterns will assist shape public health measures. For food safety policies to be complete and best practices to be shared, health authorities, food safety agencies, and schools must work together. By implementing these suggestions, people who are involved can make food safety and public wellness much better.

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