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Research

Antibiotic resistance profile of bacteria isolates from ready to eat snacks in Abraka, Delta State.

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Abstract

Ready to eat foods are foods ingested without further processing, which may include snacks, dried meat, fish and cereal based foods. The purpose of study was to determine the antibiotic resistance profile of ready to eat snacks sold in Abraka. Twenty samples of ready to eat snacks were purchased from four different locations in Abraka, Delta State.Microbial quality of snacks was determined using different bacteriological media and isolates identified using morphological and biochemical tests. Antibiotic susceptibility testing was carried out using modified Kirby-Bauer disc diffusion method. The total viable count obtained from the snacks ranged from 1.7×10^{5}- 3.5×10^{5} cfu/g which was within the marginal tolerance level of 10^{4}-$<10^{6}$cfu/g for such foods. Bacteria isolated were *Staphylococcus aureus, Salmonella* spp. *Bacillus* spp, *Enterococcus* spp and *Escherichia coli*. Resistance profile of *S. aureus* varied, however highest resistance was recoded for rifampicin (88.89%). *Escherichia coli* strains were (100%) resistant to augmentin, sparfloxacin, tarivid, streptomycin, chloramphenicol and septrin. Other organisms showed resistance to more than three antibiotics. Detection of pathogenic organisms in snacks shows the need for hygienic practice during preparation and vending. Bacterial isolated from snacks showed multidrug resistance profile.

Keywords: antibiotics, bacteria, resistance profile, ready-to-eat, snacks

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Introduction

Ready to eat snacks are foods that are consumed in the same state as were sold (Kigigha, 2012) and excludes nuts in the shell, whole raw fruits and vegetables that are intended for hulling, peeling or washing by the consumer. Some ready to eat foods are regarded as potentially hazardous as they are capable of supporting the growth of pathogens. Such food must be kept at certain temperatures and conditions to minimize the growth of any pathogens that may be present in the food or to prevent the formation of toxins in the food (Oranusi, et al ., 2011). Snacks like meat pie, eggroll, fish pie, buns, chin-chin, doughnut, fried fish, moi-moi and bean cakes are high energy foods consumed by all age groups, especially youths. Snacks contain nutrients like carbohydrates, proteins, fats, oil and water. The nature of snacks and their method of preparation, involving extensive handling and use of utensils, make them prone to contamination and cross contamination from environment, human activities and distribution facilities.

The microbial agents that cause foodborne illness may include *Salmonella*, pathogenic strains of *Escherichia coli*, *Bacillus* spp, *Clostridium* spp, hepatitis A and E, mold and yeasts. The incidence of foodborne diseases is rapidly increasing due to unhygienic methods of processing foods (WHO, 2015). Most etiologic agents of foodborne diseases have developed resistance to commonly used antibiotics. Transmission of antibiotic resistance genes to pathogenic organisms have resulted to higher mortality and morbidity rates (Economou & Gousia, 2015).

The dissemination of antibiotic resistant pathogens from food is an emerging public-health threat (Lin et al., 2017). Snacks which are ready to eat foods do not need further thermal processing before ingestion could be vehicle for the transfer and spread of antibiotic-resistant microorganisms. Therefore, study was undertaken to investigate the antibiotic resistance profile of bacteria isolated from snacks sold in Abraka.

Materials and methods

Sample collection

Meat pies. Fish pies, buns, sausages, and egg rolls were randomly purchased from four different busy locations in Abraka. A total of twenty samples were packaged in sterile bags and transported to the Microbiology Laboratory, Delta State University for further study.

Isolation and identification of microorganisms

One gramme of homogenated sample was introduced into 9ml of sterile distilled water and serially diluted. One milliliter of 10^{-1}-10^{8} dilutions were introduced into molten sterilized nutrient agar, MacConkay agar and mannitol salt agar. Plates were incubated at

37^{o}C for 18-24 hrs. Pure isolates were subjected to Gram staining and biochemical tests. The results obtained were interpreted using Bergey's manual of systematic bacteriology (Holt et al., 1993).

Antimicrobial susceptibility testing

Standardized cultures of various bacteria were streaked onto freshly prepared Mueller Hinton agar using a sterile wire loop. The inoculum was standardized by introducing five colonies of each test organism into sterilized Mueller Hinton broth and incubated at 37^{o}C for 6 hours. The turbidity of the actively growing broth culture was adjusted with sterile broth to achieve a turbidity equivalent to that of 0.5 McFarland standard (Cheesebrough,2004). The antibiotic discs were placed on the agar plates and incubated for 24hours at 37^{o}C. The antibiotics included ciproflox (10µg), norfloxacin (10µg), gentamycin (10µg), amoxil (20µg), streptomycin (30µg), rifampicin(20µg), erythromycin (30µg), chloramphenicol (30µg), ampiclox (20µg), levofloxacin(20µg), septrin (30µg), sparfloxacin(10µg), amoxacillin (10µg), augumentin (10µg), perfloxacin 30µg) and tarivid (10µg). A meter rule was used to measure the diameter of each zones in millimeters (Cheesebrough, 2004).

Results

Bacteria Isolated from snacks

Ready to eat snacks used in this study included meat pies, fish pies, sausage rolls, egg rolls and buns. A total of 35 bacteria were isolated and identified from the ready to eat snacks. The bacteria isolated from snacks included *Staphylococcus aureus* 9(25.7%) *Salmonella* spp. 8(22.9%), *Enterococcus* spp. 7(20.0%), *Bacillus* spp. 6(17.1%) and *Escherichia coli* 5 (14.3%). Total aerobic count obtained from the floury products ranged from 1.7×10^{5}- 3.5×10^{5}cfu/g while total mean coliform counts for meatpie and fish roll were 1.2×10^{5} cfu/g and 1.0×10^{5} cfu/g respectively as presented in table 1.

Table 1: Bacteria isolated from Ready -to-eat sold in Abraka

			Bacteria species					
Type of snack	Mean Total Aerobic Count (cfu/g)	Mean Coliform Count (cfu/g)	*Staphylococcus aureus*	*Bacillus spp.*	*Enterococcus spp.*	*Escherichia coli*	*Salmonella spp.*	Total organisms from snacks
Meat pie (n=4)	3.4×10^5	1.2×10^5	1 (12.5%)	1(12.5 %)	0 (00.0%)	3 (37.5%)	3 (37.5%)	8 (22.9%)
Fish pie (n=4)	3.0×10^5	1.0×10^5	1 (14.35%)	0 (0.0%)	2 (28.6%)	2 (28.6%)	2 (28.6%)	7 (20.0%)
Eggroll (n=4)	1.7×10^5	-	2 (28.6%)	1(12.5 %)	3 (42.9%)	0 (00.0%)	1 (14.3%)	7 (20.0%)
Buns (n=4)	3.0×10^5	-	2(40.0%)	1(12.5 %)	1 (20,0%)	0 (00.0%)	1 (20.0%)	5 (14.2%)
Sausage (n=4)	3.5×10^5	-	3 (37.5%)	3(37.5 %)	1 (12.5%)	0 (00.0%)	1 (12.5%)	8 (22.9%)
Total 20			9 (25.7%)	*6*(17.1 %)	7 (20.0%)	5 (14.2%)	8(22.9%)	35 100.0%)

Table 2 : Antibiotic Susceptibility Results of *Escherichia coli* and *Salmonella* spp.

Isolated from ready- to- eat snacks in Abraka

Families of antibiotics	Antibiotics tested	*Escherichia coli* n=5 (%)		*Salmonella* spp.n=8 (%)	
		R	S	R	S
Aminoglycoside	CN	4(80.00)	1(20.00)	8(100.00)	0
	S	5(100.00)	0	8(100.00)	0
Chloramphenicol	CH	5(100.00)	0	5(62.50)	3(37.5)
Quinolones	CPX	4(80.00)	1(20.00)	4(50.00)	4(50.00)
	PEF	4(80.00)	1(20.00)	8(100.00)	0
	SP	5(100.00)	0	6(75.00)	2(25.00)
	OFX	5(100.00)	0	6(75.00)	2(25.00)
B-lactams	PN	3(60.00)	2(40.00)	6(75.00)	2(25.00)
	AU	5(100.00)	0	8(100.00)	0
Sulfonamides	SXT	5(100.00)	0	6(75.00)	2(25.00)

R=Resistance, S= sensitive, PN-Amoxicillin, AU-Augumentin, CN-Gentamycin, SP-Sparfloxacin, OFX-Tarivid, S-Streptomycin, CH-Chloramphenicol, SXT-Septrin, CPX-Ciprofloxacin, PEF-Perfloxacin.

Table 3: Antibiotic Susceptibility Results of *Staphylococcus aureus*, *Bacillus* spp. and *Enterococcus* spp. Isolated from ready -to- eat snacks in Abraka

Families of antibiotics	Antibiotics tested	*S. aureus* n=9 (%)		*Bacillus* spp. n=6(%)		*Enterococcus* spp. n=7 (%)	
		R	S	R	S	R	S
Aminoglycosides	CN	5(55.56)	4(44.44)	5(83.33)	1(16.67)	7(100.00)	0
	S	4(44.44)	5(55.56)	6(100.00)	0	7(100.00)	0
Macrolides	E	3(33.33)	6(66.67)	5(83.33)	1(16.67)	5(71.43)	2(28.57)
Chloramphenicol	CH	5(55.56)	4(44.44)	6(100.00)	0	4(57.14)	3(42.86)
Quinolones	CPX	4(44.44)	5(55.56)	6(100.00)	0	6(85.71)	1(14.29)
	NB	6(66.67)	3(33.33)	6(100.00)	0	6(85.71)	1(14.29)
	LE	5(55.56)	4(44.44)	6(100.00)	0	7(100.00)	0
B-lactams	AML	7(77.78)	2(22.22)	4(66.67)	2(33.33)	7(100.00)	0
	APX	5(55.56)	4(44.44)	4(66.67)	2(33.33)	7(100.00)	0
Rifamycins	RD	8(88.89)	1(11.11)	6(100.00)	0	7(100.00)	0

R=Resistance, S= sensitive ,AML-Amoxil, APX-Ampiclox, E-Erythromycin, CN-Gentamycin, LE-Levofloxacin, S-Streptomycin, CH-Chloramphenicol, CPX-Ciprofloxacin, NB-Norfloxacin, RD-Rifampicin

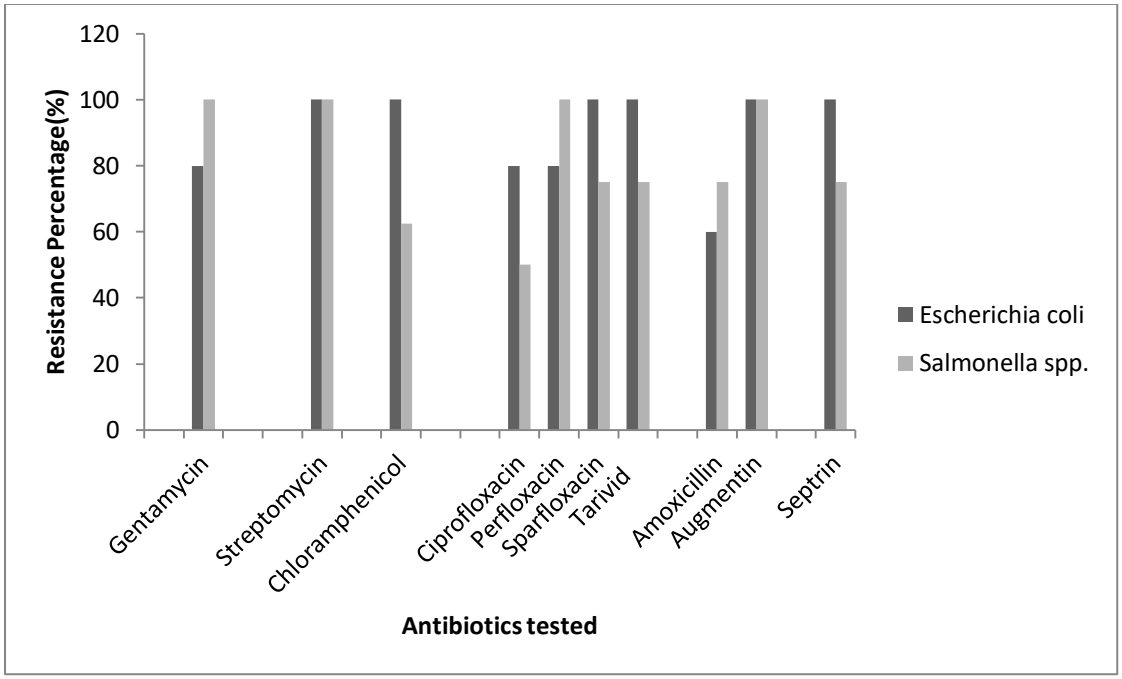

Figure 1; Resistance profile of *Escherichia coli* and *Salmonella* spp.

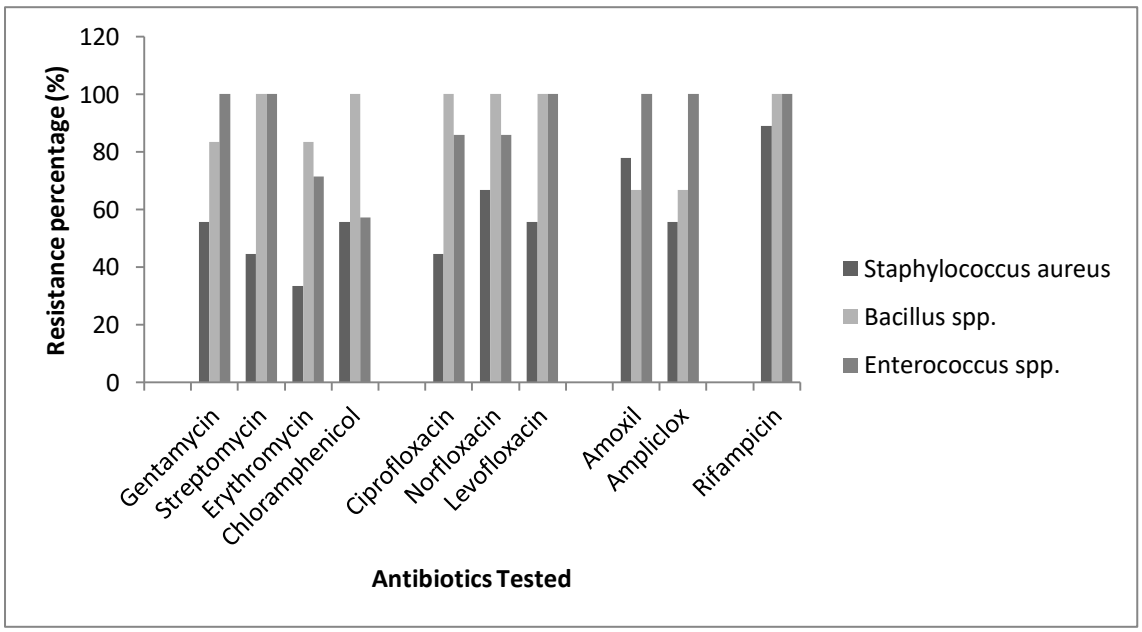

Figure 2: Resistance profile of *Staphylooccus aureus, Bacillus* spp. and *Enterococcus* spp.

The results of antibiotics susceptibility pattern of bacteria isolated from snacks are shown in tables 2 and 3. Table 2 shows susceptibility results of *Escherichia coli* and *Salmonella* spp. isolated from ready to eat snacks in Abraka. *Escherichia coli* strains were susceptible to ampicillin while *Salmonella* spp. were sensitive to ciprofloxacin.

Table 4 presents the antibiotic susceptibility results of *S. aureus, Bacillus* spp *and Enterococcus* spp. *S aureus* strains showed varying susceptible to erythromycin and streptomycin. While *Bacillus* spp were susceptible to amoxil and ampliclox (B-lactam).

Figure 1 presents resistance profile of *Escherichia coli* and *Salmonella* species. Results showed that *Salmonella* species were resistant to aminoglycosides and quinolone . *Escherichia coli* strains were resistant to antibiotics from the following classes aminoglycosides, chloramphenicol, quinolone, sulfonamide and B-lactams.

Figure 2 shows the resistance profile of *Staphylococcus aureus, Bacillus* spp , and *Enterococcus* spp. *Staphylococcus aureus* showed highest resistance to rifampicin while *Bacillus* spp. were resistant to chloramphenicol and the quinolones. *Enterococcus* s and *Bacillus* pecies were resistant to gentamycin, amoxil , ampliclox, streptomycin, levofloxacin and rifampicin respectively.

Discussion

Antibiotic resistance profile of ready to eat snacks from four different sites in Abraka, Delta State were assessed. The total aerobic counts in this study was within tolerable limit accepted, which was similar to the findings of Oranusi et al. (2011) for eggrolls and sausages obtained from snacks bar in Ota, Ogun State which mean microbial loads were 1.8×10^5 and 5.8×10^5 respectively. Contrary, though, to reports from previous researchers which were within the acceptable range of 2.5×10^4 cfu/g (Oranusi & Braide, 2012; Adebisi & Oyetayo, 2019). According to microbiological guideline for ready to eat food placed in the market, the total aerobic count at the borderline for bakery and confectionery products without dairy creams, and powdered foods was 10^4-$<10^6$ cfu/g (HPA, 2009). Results obtained from study was within the borderline limit. This implies that the snacks were of acceptable standard according to the guidelines. Likewise, findings from fish pies, buns and egg rolls were all within marginal limits in this study. The nutritional content of these snacks may have been responsible for moderate bacterial count obtained from the snacks. The total aerobic colony count is an indicator of quality and cannot contribute to safety assessment of ready to eat food but could be used to assess quality and remaining shelf life of food (Health protection Agency, 2009; CFS, 2014).

Salmonella spp. and *Bacillus* spp. were detected in some of the snacks. The source of organisms could have been from ingredients used for preparation. The presence

of *Escherichia coli* in eggroll and fish pie, indicated feacal contamination which show poor hygiene during snack preparation. This findings contradicts previous reports for meat pies where *E. coli* was not detected (Oranusi & Braide, 2012). However, other studies reported the presence of *E. coli* and *Staphylococcus aureus* from ready to eat snacks (Oje *et al*., 2018; Nwachukwu &Nwaigwe, 2013) thereby supporting the finding of this study. The presence of *S. aureus* in ready to eat snacks may be from the environment and human (Achi and Madubuike,2007; Acco et al., 2003). Furthermore, previous report showed that human carriage of *S. aureus* in Nigeria is high (Paul et al ., 1982). The susceptibility result for *S. aureus* were as follows streptomycin (44.44%), gentamycin 55.56%), erythromycin (33.33%), chloramphenicol (55.56%), ciprofloxacin, (44.44%), norfloxacin (66.67%), levofloxacin, (55.565), Ampiclox (55.56) and rifampicin (88.89%). Achi and Madubuike (2007) findings for *Staphylococcus aureus* strains from meat and fish sausages in Umuahia showed high resistance of *S. aureus* strains to streptomycin (75.0%), gentamycin (70.8%), and amoxycillin (42.0%) but low resistance was reported for rifampicin, (4.2%), erythromycin 4.2%), ciprofloxacin (12.5%), and (0.0%) for norfloxacin and chloramphenicol respectively. These contrasting susceptibility pattern may be due to different strains of organism obtained from snacks.

In this study *Bacillus* spp. were resistant to all quinolones (norfloxacin, ciprofloxacin and levofloxacin), streptomycin and chloramphenicol and rifampicin. *Bacillus cereus* from food samples in Port Harcourt, Nigeria were resistant to norfloxacin, ampliclox and floxapem but susceptible to streptomycin, chloramphenicol, rifampicin, erythromycin, ciprofloxacin and gentamycin (Agwa et al., 2012). *Escherichia. coli* strains were resistant to augumentin, sparfloxacin, tarivid, streptomycin, chloramphenicol and septrin in this study. Similarly, *Enterococcus* spp were resistant to B-lactams (amoxil, ampliclox), gentamycin, levofloxacin, streptomycin and rifampicin. Report from another study showed that *Salmonella* spp were 100% resistant to ampicillin and 88.9% to chloramphenicol (Temesgen *et al.*,2016), in this study however, the organism was 100% resistant to augmentin, gentamycin and streptomycin. Mutation and acquisition of genes through gene transfer could be responsible for resistance of these bacteria.

Conclusion

This study showed that all strains of bacteria obtained from ready to eat snacks were resistant to streptomycin except *Staphylococcus aureus. Escherichia coli* strains *Enterococcus* spp. and *Bacillus* spp. were resistant to six antibiotics. Microbial load of meat pie, fish pie, sausage roll, egg roll and buns were of marginal tolerable level. Vendors need to be educated on more hygienic method of snacks preparation. Though the results obtained from microbial load showed

that the snacks were of acceptable marginal acceptable limit, the high resistance to antibiotics as shown by the organisms isolated from ready to eat snacks portray that snacks may serve as vector for transferring antibiotic resistance bacteria to humans, implying costlier treatment options for cases of foodborne infections or diseases. Further studies to determine the resistance pattern of foods vended in Abraka is recommended for public health enlightenment and epidemiological studies for disease prevention and improved health condition of the populace.

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