

## OCCURRENCE OF MULTIPLE DRUG RESISTANT SALMONELLA FROM SNACKS IN CALABAR, CROSS RIVER STATE, NIGERIA

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Received - 22.02.2019; Reviewed and accepted - 10.03.2019

### ABSTRACT

**Background:** This was a study to investigate the occurrence of multiple drug resistant *Salmonella* from snacks in Calabar, Cross River State, Nigeria.

**Methods:** One hundred (100) samples from eateries and streets were cultured using standard methods for *Salmonella* infections diagnosis. Antibiotic susceptibility test was carried out using agar-disc diffusion method.

**Results:** Out of 100 snacks examined with 50 each from the streets and 50 each from the eateries, 55 samples were found to harbour *Salmonella* isolates. The distribution of *Salmonella* isolates in snacks from the streets was 76% (38/50) while that of the eateries was 34% (17/50). The antibiogram of *Salmonella* showed that Ofloxacin, Ciprofloxacin, Pefloxacin, Gentamycin, Streptomycin and Cephalixin were the most sensitive while Septrin was the most resistant.

**Conclusion:** This study observed the presence of multiple drug resistant *Salmonella* from snacks in Calabar. There is therefore need for good sanitary practices and control of the use of antibiotics.

**Keywords:** *Salmonella*; antibiotics; susceptibility; resistance.

### INTRODUCTION

Infections with non-typhoid *Salmonella* enterica serovars represent an important public health problem worldwide. An estimated 1.4 million cases of salmonellosis leading to about sixteen thousand (16,000) hospitalizations and nearly six hundred (600) deaths occur each year in the United States [1]. *Salmonella* infections in humans often result from ingestion of contaminated foods such as poultry, beef, pork, eggs, milk, sea foods and fresh produce [2]. Direct contact with animals also results in transmission of *Salmonella* to human [3]. Salmonellosis in human is usually self-limiting diarrhea that does not warrant antimicrobial therapy. However, these infections can also lead to life threatening systematic infections that require effective chemotherapy [4,5].

In the last twenty (20) years, the worldwide emergence of multi-drug resistant phenotype among *Salmonella* serotypes in particular *Salmonella* serotype Typhimurium [6,7] and more recently, *Salmonella* serotype Newport is of increasing concern [3,8]. This has become a public health issue worldwide [9]. A variety of foods and environmental sources harbour bacteria that are resistant to one or more antimicrobial drugs used in human or veterinary medicine and in food animal production [10,11,12].

In a research that was conducted to isolate, characterize, identify and test for the susceptibility pattern of *Salmonella* from two hundred (200) ground meat of chicken, beef, turkey and pork purchased at three supermarkets in United States, 20% contained *Salmonella* with a total of 13 serotypes. 84% of the isolates were resistant to at least one antibiotic and 53% to at least three antibiotics [13]. This implied that there is a continuous increase in the antimicrobial resistant serotypes of *Salmonella* due to the use of antimicrobial agents in animal feeds and the indiscriminate use of the antimicrobial agents in the treatment of *Salmonella* and other bacterial infections [13].

On a daily basis, the food and animal products which are infected with *Salmonella* serotypes are used in the preparation of other food products like snacks for public consumption which has been implicated to causing many health related problems. In 2007, four (4) large multi-state outbreaks of *Salmonella* infections were investigated: an outbreak of *Salmonella* enterica serotype Tennessee caused by contaminated peanut butters; an outbreak of *Salmonella* enterica serotype Wandsworth and *Salmonella* enterica serotype Typhimurium attributed to puffed vegetable; an

outbreak of *Salmonella* enterica serotype 14, 5, 12 caused by contaminated frozen pot pies and an outbreak of *Salmonella* Paratyphi B variant Java associated with exposure to turtles [14].

In spite of the menace posed by *Salmonella* species, there is paucity of information on the prevalence, characteristics, identification and susceptibility characteristics of *Salmonella* species in snacks in Calabar. The aim of this study was to isolate, characterize and identify multiple drug resistant *Salmonella* from snacks in Calabar, Nigeria.

### METHODS AND MATERIALS

#### Study area

The study area is Calabar (Calabar Municipality and Calabar South) and it is the capital of Cross River State in South-South region of Nigeria. This city lies adjacently between latitude 4°48N and longitude 8°17E of the equator. The city is surrounded by the Calabar River in the West; Odukpani Local Government Area in the North; the Great Qua River and Akpabuyo Local Government Area in the East; and swamps, creeks and the Atlantic Ocean in the South.

#### Sample collection and processing

One hundred (100) samples of snacks with 50 each obtained from eateries and streets and immediately taken to the microbiology laboratory of the University of Calabar for analysis.

#### Pre-enrichment

Nine milliliters (9ml) of peptone water was prepared and dispensed into clean capped test tubes and was autoclaved. Upon cooling, 1g of each snack was blended into the tubes of peptone water and incubated for 24 hours at 37° C to stimulate the growth of stressed organisms [15].

#### Enrichment

Nine milliliters (9ml) of Rappaport Vasilliadis broth was prepared and dispensed into clean capped test tubes and was autoclaved. 1ml of the blended peptone broth mixture was dispensed into the tubes of Rappaport Vasilliadis broth upon cooling. It was incubated for 24 hours at 37° C to suppress the growth of

undesirable organisms and stimulate the growth of the desired ones [16].

**Isolation**

Bismuth sulphite agar was prepared in a capped Erlenmeyer flask and was uniformly heated to incipient boiling using a hot pot. Upon cooling, the agar was poured into sterile petri dishes and allowed to solidify. It was oven-dried, stored overnight in a dark refrigerator, then spread plated with 0.1ml of Rappaport Vasilliadis broth mixture. The petri dishes were incubated for 24 hours at 37° C for growth of colonies [17].

**Morphology Identification**

The morphology techniques used in this study was gram staining and motility [18].

**Biochemical Tests**

The biochemical tests employed in this study were as follows: catalase test, indole test, sugar fermentation, citrate test, urease test, methyl red test, Voges-proskauer test, and oxidase test [18].

**Antibiotic susceptibility test**

The method used in this study was Kirby-Bauer test (agar-disc diffusion method). Mueller Hinton agar was prepared with distilled water into clean capped Erlenmeyer flask and autoclaved. Upon cooling, it was poured into sterile petri dishes and allowed to solidify. With a sterile inoculating loop, the surface of the agar was entirely streaked with the isolated colony. A gram negative multiple-disc was aseptically placed on the surface of the agar and was incubated at 37° C for 24 hours. Bacteria susceptibility to the particular antibiotic disc was shown by an area of clear media where bacteria are not able to grow which surrounds the disc known as the zone of inhibition. Some disks had no zone of inhibition, indicating the resistance of the organism to such antibiotics. [17].

**Results**

The distribution of *Salmonella* isolates from snacks in Calabar is shown in Table 1. Out of 100 samples used in this study, 55 contained *Salmonella* species. Of the 55 infected samples in this study, those from the streets recorded a higher rate of infection than those from the eateries.

**Table 1: Distribution of Salmonella isolates from snacks in Calabar**

Source	No. examined	No. infected (%)
Eateries	50	17 (34)
Streets	50	38 (76)
<b>Total</b>	<b>100</b>	<b>55 (55)</b>

Table 2 presents the antibiogram of *Salmonella* species isolates from snacks in Calabar. The result shows significant percentage of the isolates was resistant to the antibiotics tested. It also revealed that all the isolates showed 100% susceptibility to Ofloxacin, Ciprofloxacin, Pefloxacin, Gentamycin, Streptomycin and Cephalixin and most resistant to Septrin.

**Table 2: Antibiogram of Salmonella isolates from snacks in Calabar.**

Antibiotics	<i>Salmonella</i> isolates (n=55)			
	Concentration (µg)	Highly sensitive (%)	Intermediate (%)	Resistant (%)
Ofloxacin	10	42 (76.4)	13 (23.6)	-
Ciprofloxacin	10	50 (90.9)	5 (9.1)	-
Pefloxacin	10	42 (76.4)	13 (23.6)	-
Augmentin	30	17 (30.9)	32 (58.2)	6 (10.9)
Gentamycin	10	30	25 (45.5)	-

		(54.5)		
Septrin	30	-	18 (32.7)	37 (67.3)
Streptomycin	30	30 (54.5)	25 (45.5)	-
Cephalixin	10	30 (54.5)	25 (45.5)	-
Nalidixic acid	30	13 (23.6)	30 (54.5)	12 (21.8)
Ampicillin	30	13 (23.6)	30 (54.5)	12 (21.8)

Table 3 presents the multiple antibiotic resistance profile of *Salmonella* isolates from snacks in Calabar. The result showed that resistance to 2 or 3 antibiotics was common among the isolates.

**Table 3: Multiple antibiotic resistance profile of Salmonella isolates from snacks in Calabar**

Antibiotics	No. of multiple antibiotic resistant <i>Salmonella</i> (%)
Resistance to 10 antibiotics	-
Resistance to 9 antibiotics	-
Resistance to 8 antibiotics	-
Resistance to 7 antibiotics	-
Resistance to 6 antibiotics	-
Resistance to 5 antibiotics	-
Resistance to 4 antibiotics	3 (13.0)
Resistance to 3 antibiotics	8 (34.8)
Resistance to 2 antibiotics	12 (52.2)
<b>Total</b>	<b>23</b>

**DISCUSSION**

Food-borne infections caused by *Salmonella* species are important public health and an economic burden to humans. The number of documented cases of these infections associated with snacks has tremendously increased.

The U.S. Food and Drug Administration in February 2007 issued a warning to consumers not to eat certain jars to Peter Pan peanut butter or Great Value peanut butter due to the risk of contamination with *Salmonella* Tennessee [19]. In March 2007, around 150 people were diagnosed with salmonellosis at a governor's reception in Krasnoyarsk, Russia. Over 1,500 people attended the ball in March 1st and fell ill as a consequence of ingesting sandwich tainted with *Salmonella* [16]. It was also reported that 150 people were sickened by chocolate cake tainted *Salmonella* produced by a major bakery chain in Singapore in December, 2007 [20].

In this study, the isolation of *Salmonella* in snacks is very disturbing due to the fact that these snacks were obtained from eateries and at various locations of the streets where many people visit every day. In Calabar, the outbreaks of snack-borne infections by *Salmonella* are seldom reported. Therefore, there is paucity of information on this form of food-borne infection transmission.

The high incidence of *Salmonella* contamination reported in this study may partly be accounted for by the use of contaminated animal-products in the preparation of the snacks. It may also be due to the use of non-potable water for processing of the snacks. It has been a great concern for the dwellers in Calabar to have potable water because many of the water sources in Calabar are contaminated.

The high *Salmonella* contaminations of snacks from the street against the eateries is an indication of the changes on the level of sanitation and hygiene observed during the preparation and packaging of the snacks from those sources.

The 100% susceptibility of the *Salmonella* isolates to Ofloxacin, Ciprofloxacin, Pefloxacin, Gentamycin, Streptomycin and Cephalixin may be attributed to the fact that these antibiotics are rarely used due to its cost in Calabar while the high resistance of the isolates to Septrin may be due to its high abuse. Resistance of

the isolates to antibiotics like Ampicillin, Nalidixic acid and Augmentin implies resistance to these antibiotics may increase with time if not effectively used.

### CONCLUSION

The study shows that there was a high incidence rate of multiple antibiotics resistant strains of *Salmonella* in snacks in Calabar. It also suggests that Ofloxacin, Ciprofloxacin, Pefloxacin, Gentamycin, Streptomycin and Cephalixin in spite of their prolonged use are still effective for the treatment of infections caused by *Salmonella* and Septrin is highly resistant to *Salmonella* infections. The result of this work also shows the extent of contamination of snacks in Calabar.

### RECOMMENDATIONS

There is need for government to establish and enforce national standards for ready to eat snacks and adhere to it strictly. The government should establish organizations that would frequently investigate and monitor ready to eat snacks sold in public places. The government should stop the indiscriminate sales of antibiotics unless prescribed by a physician after a thorough diagnosis to any infection.

The public should be enlightened on the effects of indiscriminate usage of antibiotics and various ways by which drug resistance can develop. Also, people should be educated on the level of awareness on health especially as regarding asymptomatic and carriers to prevent outbreak of food poisonings and contaminations should be increased.

Food handlers should constantly be screened. High level of hygiene and sanitation should be maintained before, during and after food processing including the packaging of the foods.

**Statement of authors' contributions:** The study was design and written by Etefia Etefia while the laboratory assistance was done by Onete Inah.

**Funding:** None

**Conflict of interest:** Not declared

**Ethical consideration:** Not required

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