



Effect of Residual Antibiotics in Snacks against *Escherichia coli* and *Staphylococcus aureus*

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Abstract

This study assessed the effect of ethanolic, hot water and cold water extracts of processed ready-to-eat snacks containing proteinous substances against *Escherichia coli* and *Staphylococcus aureus*. The snacks (shawama, Scotch egg and meat pie) were purchased from fast food hawkers in Yenagoa metropolis, Bayelsa state, Nigeria. Disk-diffusion method was used for the sensitivity testing of the various extracts and 1% Ampiclox was used for the comparison of the antibacterial effects. Results showed that ethanolic extracts have superior effect compared to cold and hot water. For hot water treatment, the *E. coli* zone of inhibition for scotch egg, shawama and meat pie were 8.38, 12.57 and 10.48mm respectively. For cold water extracts, these were 10.48, 13.62 and 8.38mm respectively. For the ethanolic extracts, the zone of inhibition for the snacks was 12.57mm (Scotch egg), 14.67mm (shawama) and 13.62mm (meat pie). For *S. aureus*, the corresponding zones of inhibition for the snacks i.e scotch egg, shawama and meat pie were 10.48, 14.67 and 8.38mm respectively (for hot water extract), 10.48, 15.71 and 12.57mm respectively (for ethanolic extract) and 10.48, 12.57 and 10.48mm respectively (for cold water extract). Ethanolic extract had higher zones of inhibition on both isolates. Also the *E. coli* had superior zone of inhibition compared to 1% Ampiclox than *S. aureus*. Analysis of variance showed that there was significant difference ($P < 0.05$) among the test organisms used in this study based on the treatments. This study showed that residual antibiotics used for proteinous sources (e.g egg, meat etc) were transferred to their final products widely consumed by several people as fast foods, which are becoming more popular with students and the working class who spent long hours from home.

Keywords: Food, Protein, Snacks, *Escherichia coli*, *Staphylococcus aureus*

1 Introduction

Food is a composite matrix that contains nutrients to enhance growth [1]. Food substances include carbohydrate, protein, lipids and water etc. Ready-to-eat foods or snacks are foods that do not require further processing prior to consumption. Most of these ready to eat foods are vended along highways, streets, markets and some public places including schools, hospitals, Motor Parks, etc. Some common ready to eat products include tomato, cabbage, ginger drink, lettuce, carrot, kunu zaki drink, akara, fried groundnut, rice and beans, fried fish, bread, zobo drink [2], and snacks such as scotch egg, shawama, meat pie etc. In developing nation like Nigeria, the consumption of snacks is high. This could be due to high rate of unemployment and failed family and community values [3]. Like fruits most ready-to-eat food are rich in nutrients, micronutrients, vitamins and fibre.

Like most ready to eat food products, Scotch egg, shawama, meat pie are sold in public places including markets, fast food, stores, restaurants etc. During their production, several natural and proteinous ingredients are used. For instance, poultry egg is used for the production of Scotch egg. The poultry industry in which poultry-meat is considered healthier than red meat is a vital source of animal proteins [4]. In the poultry industry antibiotics are used for the rearing of the birds due to several microbial diseases that infects birds. As such antibiotics and growth promoters are commonly used by poultry farmers for the control infections [4]. Also, during the processing of scotch egg, shawama, meat pie, other natural products with

antimicrobial activities such as pepper are added at varying concentrations.

E. coli is a typical example of the family *Enterobacteriaceae* which are associated with gastroenteritis. The *Staphylococcus aureus* on the other hand represents the superficial agents of infection. The study therefore meant to test antibacterial inhibition caused by residual antibiotics in some common snacks and comparing their inhibition with that of 1% broad spectrum antibiotics (Ampiclox).

2 Materials and Methods

2.1 Source of samples

Triplicate samples of the snacks (Scotch egg, meat pie and shawama) were bought from fast food hawker in Yenagoa metropolis, Bayelsa State, Nigeria and transported to the laboratory. Analysis was carried out <4 hours after purchase.

2.2 Preparation of Sample Extracts

In the laboratory, the various samples (Scotch egg, meat pie and shawama) were separately macerated in a sterile mortar and pestle. Then 10g each of the macerated samples were soaked in 100ml of solvents (ethanol, hot water and cold water) separately, which was vigorously shaken intermittently for 24 hours. Thereafter, the samples was filtered with muslin cloth followed by Whiteman No. 1

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filter paper. All extracts were preserved at 5°C in the refrigerator prior to the sensitivity testing.

2.3 Source of bacterial isolates

Test pathogenic bacteria isolates i.e *S. aureus* and *E. coli* used for the study were obtained from Glory land Clinic, Yenegoa, Bayelsa State of Nigeria. The purity of the isolates was tested by subculturing and they were preserved in nutrient agar slants at 5°C in the refrigerator prior to use.

2.4 Impregnation of filter-paper discs with the extracts and Antibacterial assessment

The disk-diffusion technique previously described by Kigigha and Atuzie [5], Kigigha and Charlie [6], Kigigha and Onyema [7] were used to test the antibacterial activity of the different extracts using 10 mm discs. Whatman No. 1 filter paper was used to prepare the antibiotic discs using cock borer. The solution containing the extracts (0.1ml each) were impregnated on the disc using sterile pipette and air-dried. 1% Ampiclox solution (which served for comparison) was also impregnated on filter-paper discs. Flame-sterilized and cooled pair of forceps was used for the transfer of the discs separately. The different treatments were placed separately on nutrient agar plates with lawn spread growth of the test isolates. The filter paper impregnated with 1% Ampiclox solution was also placed separately on the lawn spread test isolates. The discs were well spaced out on the agar plates to avoid overlapping of the zones of inhibition. The plates were

incubated at 37°C for 72 hours to observe and measure the zones of inhibition.

2.5 Statistical Analysis

Data was analyzed using *Zigma-Stat 32* Statistical package. Where normality and homoscedasticity were satisfied, data from treatment were analyzed using one-way analysis of variance (ANOVA). Comparison for treatments was analyzed using *Tukey* tests at $P=0.05$.

3 Results and Discussion

Figure 1 presents the antibacterial effects of the snacks on some pathogenic microbes which was observed for hot water scotch egg extracts were 8.38 and 10.48mm for *E.coli* and *S. aureus* respectively; for hot water shawama extracts were 12.57 and 14.67mm for *E.coli* and *S. aureus* respectively and for hot water meat pie extracts were 10.48 and 8.38mm for *E.coli* and *S. aureus* respectively. The various ethanolic extracts of the snacks were 12.57 and 10.48mm for *E.coli* and *S. aureus* respectively (scotch egg), 14.67 and 15.71mm for *E.coli* and *S. aureus* respectively (shawama) and 13.62 and 12.57mm for *E.coli* and *S. aureus* respectively (meat pie). Also the cold water extracts for the various snacks was 10.48 and 13.62mm for *E.coli* and *S. aureus* respectively (scotch egg), 10.48 and 12.57mm for *E.coli* and *S. aureus* respectively (shawama) and 8.38 and 10.48mm for *E.coli* and *S. aureus* respectively (meat pie).

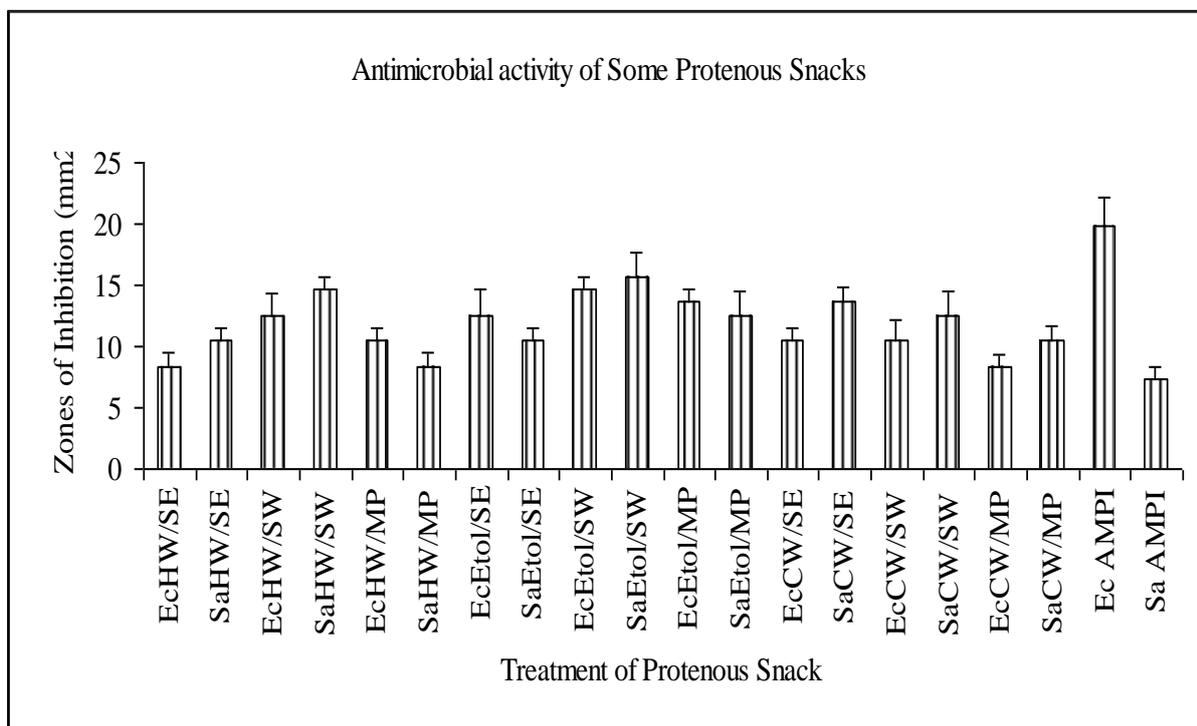


Fig 1: Zone of Inhibition for some fully processed snacks (shawama, meat pie and Scotch egg). (Ec= *E.coli*, Sa = *S. aureus*, SE = Scotch egg, SW= Shawama, MP=Meat pie, Etol =Ethanol extracts, CW = Cool water extracts ; HW= Hot water extracts); n=3

The zone of inhibition for the 1% Ampiclox were 19.90 and 7.33mm for *E.coli* and *S. aureus* respectively. Basically there was significant difference ($p<0.05$) among the various treatments in both bacterial isolates. This could be due to the various composition and level of incorporation of spices/preservatives during their processing. Again, the level of heating the products are

subjected to could have also contributed to the antibacterial efficacy of the various snacks under study. The results fluctuate for instance, *S. aureus* was higher in scotch egg and shawama samples and lower for meat pie (hot water extracts), *E. coli* have superior zone of inhibition for scotch egg and meat pie but lower for shawama) and cool water extract were consistent showing

that *S. aureus* has superior zone of inhibition than *E. coli*. However, Ampiclox has lower and highest zone of inhibition for *S. aureus* and *E. coli* respectively. The trend of ampiclox having lesser effect in *S. aureus* when compared to *E. coli* in agreement with the findings of several authors on different substrates [5 – 7]. On the overall, ethanol had higher zone of inhibition when compared to other extracts (hot water and cold water). The trend in this study was in conformity with findings of Masih *et al.* [8], Ere *et al.* [9], Akintobi *et al.* [10] on antibacterial effects of different plant species on some microbes including the ones used in this study. Opoku and Akoto [11] has attributed the presence of insoluble active compound found in cold water and or denaturation of the active constituents by the hot water extraction process as potential reason why they have lower zone of inhibition.

The antibacterial potentials in the snacks (shawama, scotch egg and meat pie) could stem from the fact that livestock is an ingredient in the production of these snacks. Poultry products including its meat (used for the processing of the meat pie and shawama depending on choice), egg (used for the processing of the scotch egg) could be source of the antibacterial agent in the products. Sometimes, the preservative or spice added to the food products could also be potential antibiotics in the snacks.

4 Conclusion

This study evaluated the antimicrobial effects of some fully processed snacks such as scotch egg, shawama and meat pie prepared with poultry products on bacterial isolates. The study found that the snacks have antibacterial effects against common enteric and superficial etiologic agents thus *E. coli* and *S. aureus* respectively. This is an indication that drug-resistant in normal human flora could be elated through eating of snacks. This also highlighted the need to cure farm animals and their products of residual antibiotics before they are sold to public. This could be achieved through the use of prebiotics and probiotics and botanicals in the rearing of livestock. Also it appears to be no legislation in Nigeria in this respect.

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