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# Microbial distribution and antimicrobial effects of AgZnO and AgTiO nanoparticles (NPs) against microorganisms isolated from floury snacks sold in FUTA


## Abstract

The microbial distribution and antimicrobial effect of AgZnO and AgTiO nanoparticles against microorganisms isolated from floury snacks sold in Federal University of Technology, Akure (FUTA) was investigated. Three common floury snacks viz; meat pie, eggroll and puff-puff were collected from these vendors and examined for microbial load and types using standard methods. The microbial load recorded ranged from $1.2x10^2$ to $1.8x10^4$ cfu/g. The microbial load of puff-puff was the lowest ($1.2x10^2$–$1.9x10^3$ cfu/g). The microorganisms isolated from these products include, *Staphylococcus aureus, Staphylococcus epidermidis, Bacillus* spp, *Serratia marcescens, Aspergillus niger* and *Aspergillus fumigatus*. The microbial isolates showed varying degree of resistance to commercial antibiotics while the AgZnO and AgTiO nanoparticles were able to inhibit all the isolates with zones of inhibition ranging from 9mm to 20mm at 100mg/mL. The results from this study revealed that AgZnO and AgTiO nanoparticles (NPs) has significant antimicrobial activity on microorganisms isolated from floury snacks, however, the issue of safety is still very crucial.


**Keywords:** antimicrobial, nanoparticles (NP), floury, snacks, microorganisms

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## Introduction

Street foods are edibles prepared by vendors for immediate consumption at the point of sale. These foods could be raw or cooked, hot or chilled and can be consumed without further processing.[1] Floury snacks such as meat pie, puff-puff, sausage, and burger form major part of these readymade street foods. Street foods are common in Nigeria and many developing countries. They are prepared and sold in public places such as schools, markets, pupil school's gates, campuses, interstate highways and stalls at corner of the streets to attract the consumers. Fast food enterprise is one of the major businesses that contribute to the socio-economic development in many countries.[2]

These street foods and fast foods had been the choice of people in the urban areas because the food is ready-to-eat, cheap, convenient, sweeten with a different flavor and accessible as immediate want.[3–5] However, the preparation of some of street foods do not meet the sanitary qualities and specification of food safety bodies.[6] Street foods have partially alleviated the problem of food insecurity and hunger by its steady availability but; the major concerns is its hygienic status during production and marketing system.[7] The incidence of foodborne diseases is increasing rapidly due to unhygienic methods of processing food.[8] The following microorganisms *Staphylococcus aureus, Escherichia coli, Salmonella typhi, Shigella dysenteriae, Klebsiella pneumoniae, Proteus vulgaris, Enterobacter aerogenes, Streptococcus lactis, Pseudomonas aeruginosa, Bacillus* spp, *Vibrio parahaemolyticus, Saccharomyces cerevisiae, Aspergillus* spp, *Penicillium* spp, *Rhizopus stolonifer, Mucor mucedo* and *Candida albicans* were isolated from street foods sold in Akure metropolis.[7] The authors also reported that the isolates showed varying degree of resistance to commercially available antibiotics.

In the last three decades, the issue of antibiotic resistance has taken the centre stage. This is because most microbial etiologic agents of foodborne diseases have developed resistance to commonly used antibiotics.[9] It has been reported that the transmission of antibiotic resistance genes to another pathogenic organism has contributed to food illnesses, higher morbidity and mortality rates.[10] There is a continuous search for alternative antimicrobial agent that will be more effective than commonly available antimicrobial agent. Nanomolecules are considered as an alternative to conventional antimicrobial agents. Nanomolecules can be synthesized from polymers, lipids and metals.[11] The present study was therefore designed to investigate the microbial quality and susceptibility pattern of isolates from floury snacks to AgZnO and AgTiO nanoparticles (NPs).

## Materials and methods

### Sample collection

Ready to eat floury food samples such as egg roll, meat pie, puff-puff were collected from South gates, Oba-Kekere and Oba-Nla sales point at the Campus of The Federal University of Technology, Akure. Collection was done in the morning and at evening. Food samples were aseptically collected from October 2017 to March 2018. The food samples in ice bag were transferred to the laboratory for immediate analysis.

### Isolation of microorganisms

Floury food samples collected were homogenized, and one gram of each homogenate was weighed out into 10 ml of sterile water as a stock solution. Serial dilution was carried out to obtain appropriate aliquot. Serially diluted sample (1.0 ml) was transferred into Petri dish containing Plate Count Agar (PCA, Lab M) for bacteria and Potato

Dextrose Agar (PDA, Lab M) for fungi. The plates were incubated at 37°C for 24 h and 48 h for bacteria and fungi, respectively. At the end of the incubation period, colonies were counted using the colony counter (TT-20, Techmel and Techmel, USA). The counts for each plate were expressed as colony forming unit per gram (CFU g-1) for bacteria and spore forming unit per gram (SFU g-1) for fungi. Microbial growth from the plate was subcultured to obtain pure cultures and these were kept at 4°C for further study.

## Identification of microorganisms

For the preliminary identification was carried out using Gram stain technique and biochemical tests[12,13] and the results obtained were interpreted according to Bergey's Manual of Systematic Bacteriology.[14] Identification of fungal isolates was carried out by staining with lactophenol blue and examined under a microscope. The feature characteristics of fungi were used in their identification.[15]

## Antibiotic sensitivity test

Antibiotic susceptibility test was carried out using the agar disc diffusion method following the recommendation of the Clinical and Laboratory Standards Institute.[16] The inoculum of the isolates obtained from the floury foods was prepared from 18 h old broth culture of each isolate and their absorbance was adjusted to 0.5 McFarland equivalent. Inoculum size (0.1mL) was spread on Mueller-Hinton agar and the antibiotic discs were placed at the equidistance of the plate. The antibiotics used include amoxicillin (30µg), ofloxacin (10 µg), streptomycin (10 µg), chloramphenicol (30 µg), gentamycin (10 µg), pefloxacin (10 µg), cotrimoxazole (30 µg), ciprofloxacin (10 µg), erythromycin (10 µg) and tetracycline (25 µg) obtained from Abtek Biological Ltd, Liverpool, L9 7AR, UK. The zones of inhibition were measured and interpreted according to CLSI (2012). The fungal isolates were tested against antifungal drugs, namely ketoconazole (15 µg), fluconazole (25 µg) and nystatin (1µg). This was done according to the standard methods described by CLSI (2009). Already prepared green synthesized AgZnO and AgTiO nanoparticles (NPs) were obtained from Prof. A.E. Okoronkwo of Department of Chemistry, Federal University of Technology, Akure, Nigeria. Antimicrobial activity of nanoparticles was checked against microbial isolates by agar well diffusion assay.[17]

# Results and discussion

Most food vendors that are involved in the sale of street foods lack knowledge of basic food hygiene.[18] Hence, foods served by them are suspect in terms of microbial quality. It has also been observed that food-handlers' hygienic status is a major factor contributing to the poor microbiological quality and safety of street foods.[19] Hence, the issue of the microbial quality and antimicrobial sensitivity pattern of isolates from floury snacks patronized by most students of The Federal university of Technology, Akure is germane. Results from the microbial load of the floury snacks revealed that meat pie has the highest load ($8.0x10^2$ to $1.5x10^4$ cfu/g). The bacterial load recorded for floury snacks collected in the evening was slightly higher than in the morning (Table 1). This same trend was also observed in fungal count in the floury snack collected in the morning and in the evening (Table 2). The microbial load of the floury snacks ranged from $1.2x10^2$ to $1.8x10^4$ cfu/g. This is closely related to the microbial load of $1.2x10^2$ to $1.8x10^4$ cfu/ml for total aerobic, coliform, yeast and mould counts in foods served in elementary.[20]

**Table 1** Mean bacterial load (cfu/g) of floury snack

| Source | Food Sample | Morning | Evening |
|---|---|---|---|
| | Puff puff | $4.0x10^2$ | $1.2 x10^3$ |
| South Gate FUTA | Egg roll | $2.2 x10^3$ | $6.2 x10^3$ |
| | Meat pie | $4.2 x10^3$ | $9.5 x10^3$ |
| | Puff puff | $3.8x10^2$ | $1.6x10^3$ |
| Oba-Kekere FUTA | Egg roll | $2.2 x10^3$ | $6.2 x10^3$ |
| | Meat pie | $4.0 x10^3$ | $9.8 x10^3$ |
| | Puff puff | $8.0x10^2$ | $1.2x10^3$ |
| Oba-Nla FUTA | Egg roll | $2.7 x10^3$ | $9.2 x10^3$ |
| | Meat pie | $4.5 x10^3$ | $1.5 x10^4$ |

**Table 2** Mean fungal load (cfu/g) of floury snack

| Source | Food Sample | Morning | Evening |
|---|---|---|---|
| South Gate FUTA | Puff puff | $1.2x10^2$ | $1.6 x10^2$ |
| | Egg roll | $1.6 x10^2$ | $1.6 x10^4$ |
| | Meat pie | $2.5 x10^3$ | $1.9 x10^4$ |
| Oba-Kekere FUTA | Puff puff | $1.0x10^2$ | $1.9x10^3$ |
| | Egg roll | $1.6 x10^3$ | $1.6 x10^4$ |
| | Meat pie | $1.2 x10^3$ | $9.8 x10^4$ |
| Oba-Nla FUTA | Puff puff | $1.0x10^2$ | $1.6x10^3$ |
| | Egg roll | $1.2 x10^2$ | $1.6 x10^3$ |
| | Meat pie | $4.5 x10^3$ | $2.6 x10^4$ |

The following microorganisms were isolated from the floury snacks viz; *Staphylococcus aureus, Staphylococcus epidermidis, Bacillus* sp, *Serratia marcescens, Aspergillus niger* and *Aspergillus fumigatus*. *Staphylococcus aureus* has highest percentage of occurrence being present in all the samples collected in the morning and evening (Table 3). The microorganisms isolated in this study include *Staphylococcus aureus, Staphylococcus epidermidis, Bacillus* sp., *Serratia marcescens, Aspergillus niger* and *Aspergillus fumigatus*. Similar microorganisms had been isolated from ready to eat foods by other researchers.[7,21] *Staphylococcus aureus* has the highest level of occurrence and was isolated in all the samples collected in the morning and also in the evening. *Staphylococcus aureus* is known to have the following properties: resistance to heat, drying and radiation.[22] These properties could make it survive some of the processing stages during food preparation. Moreover, it is a common organisms found on the skin of human. It can therefore be transferred from vendors to the food. *S. aureus* is an important foodborne pathogen due to its ability to produce heat stable and potent exotoxin; a common food poisoning agent. *Bacillus* sp. and *Aspergillus* spp were also prominent microbial isolates from the floury food. These isolates, *Bacillus* sp. and *Aspergillus* spp, are spore and could have survived heat processing as a result their heat resistant spores.[23] Moreover, *Bacillus* sp. and *Aspergillus* spp. are common environmental contaminants.

**Table 3** Distribution of microbial isolates in floury snacks

| Floury snack | Morning sample | Evening sample |
|---|---|---|
| Puff-puff | *Staph. aureus*, *Aspergillus fumigatus*. | *Staph. aureus*, *Bacillus sp.*, *Aspergillus fumigatus*. |
| Meat pie | *Staph. aureus*, *Staph. epidermidis*, *Aspergillus niger*, *Bacillus cereus*. | *Staph. aureus*, *Staph. Epidermidis*, *Aspergillus niger*, *Bacillus cereus*, *Serratia marcescens*. |
| Egg roll | *Staph. aureus*, *Staph. epidermidis*, *Aspergillus fumigatus*, *Bacillus cereus*. | *Staph. aureus*, *Staph. Epidermidis*, *Aspergillus niger*, *Bacillus cereus*. |

Commercial antibiotics used displayed varying inhibitory zones ranging from 18mm to 22mm against the microbial isolates from floury snack (Table 4). Ciprofloxacin and pefloxaxin exhibited significant inhibitory effect against the isolates while ampicillin, amoxicillin and septrin were unable to inhibit the isolates. However, AgZnO and AgTiO nanoparticles (NPs) exhibited significant antimicrobial effect against the isolates with zones of inhibition ranging from 9mm to 20mm (Table 5). *Staphylococcus aureus, Staphylococcus epidermidis* and *Bacillus* spp were the most susceptible to the antimicrobial effect of the NPs. The microorganisms isolated from floury snacks also showed significant resistance to commonly used antibiotics such as ampliclox, amoxicillin and septrin. Antibiotic resistance of microorganisms isolated from vended and street foods sold in Lagos and Akure respectively have been reported.[7,24] Marked resistance of microorganisms to commonly used antibiotics such as amoxicillin, tetracycline, cotrimoxazole, gentamycin, chloramphenicol, ciprofloxacin and streptomycin has been associated with co-existence of resistant genes with mobile elements such as plasmid, transposons and integrons.[25,26] Transfer of resistance gene in food products and the environment had been linked to several human activities such as use of antibiotics in farming to produce some edible foods.[27]

**Table 4** Antibiotic sensitivity pattern of microbial isolates from floury snacks to commercial antibiotics Antibiotics/ Zone of Inhibition (mm)

| Isolates | CN | Ery | Sep | amo | Cipro | Co | Pef | amp | Gen | Tetr |
|---|---|---|---|---|---|---|---|---|---|---|
| *Bacillus spp.* | 4 | 8 | 0 | 0 | 22 | 4 | 20 | 0 | 4 | 12 |
| *Serratia marcesens* | 4 | 12 | 0 | 0 | 18 | 6 | 20 | 0 | 6 | 10 |
| *Staphyloccocus aureus* | 6 | 12 | 0 | 0 | 22 | 8 | 18 | 2 | 4 | 14 |
| *Staphyloccocus epidermidis* | 6 | 12 | 0 | 0 | 20 | 8 | 20 | 0 | 4 | 10 |

CN, Chloramphenicol; Ery, Erythromycin; Sep, Septrin; Amo, Amocicillin; Cipro, Ciprofloxacin; Co, Cotrimoxazole; Pef, Perfloxacin; Amp, Ampiclox; Ery, Erythromycin; Tetr, Tetracycline

**Table 5** Inhibitory zone (mm) of AgZnO and AgTiO nanoparticles (NPs) at 100mg/mL against microbial isolates from floury snacks

| Isolates | AgZnO NP | AgTiO NP |
|---|---|---|
| *Bacillus spp.* | 18 | 16 |
| *Serratia marcesens* | 15 | 14 |
| *Staphyloccocus aureus* | 20 | 18 |
| *Staphyloccocus epidermidis* | 18 | 15 |
| *Aspergillus niger* | 11 | 10 |
| *Aspergillus fumigatus* | 10 | 9 |

The green synthesized nanoparticles, AgZnO and AgTiO nanoparticles (NPs) inhibited all the microbial isolates from floury snacks. NPs had been reported to possess broad-spectrum antibacterial properties against both Gram-positive and Gram-negative bacteria.[28] ZnO NPs were found to inhibit *Staphylococcus aureus*, and Ag NPs exhibit concentration-dependent antimicrobial activity against *Escherichia coli* and *Pseudomonas aeruginosa*.[29] In a recent study,[30,31] respectively reported inhibition of some microorganisms by AgTiO and AgZnO NP. Three mechanisms of action had been proposed for the antimicrobial action of NPs. These are oxidative stress induction;[32] metal ion release[33] and non-oxidative mechanisms.[34] These three types of mechanisms can occur simultaneously.[28]

Conclusively, the results from this study revealed that AgZnO and AgTiO nanoparticles (NPs) significantly inhibit bacterial and fungal isolates obtained from floury snacks sold around and within the campus of The Federal University of Technology. This NPs may be exploited in the preservation of these snacks after the appropriate *in-situ* safety assays might have been carried out.

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## Conflicts of interest

Authors declare that there is no conflict of interest.

## References

1. Tsang D. *Microbiological guidelines for ready to eat food*. Hong Kong: Road and environmental hygiene Department; 2002. 115–116 p.
2. Rane S. Street vended food in developing world: hazard analyses. *Indian J Microbiol*. 2011;51(1):100–106.
3. Mensah P, Yeboah-Manu D, Owusu-Darko K. Street foods in Accra, Ghana: How safe are they? *World Health Organisation Bulletin*. 2002;80(7):546–554.
4. Oranusi US, Braide W. A study of ready-to-eat foods vended on highways, Onitsha-Owerri, South West Nigeria. *Int Res J Microbiol*. 2012;3(2):66–71.
5. Rath CC, Patra S. Bacteriological quality assessment of selected street foods and antibacterial action of essential oils against food borne pathogens. *Internet J Food Safety*. 2012;14:5–10.
6. Food and Agricultural Organization /World Health Organization (FAO/ WHO). Basic steps to improve safety of street-vended food, International Food Safety Authorities Network (INFOSAN) Information Note No. 3/2010 - Safety of street-vended food; 2010.

7. Ogidi CO, Oyetayo VO, Akinyele BJ. Microbial Quality and Antibiotic Sensitivity Pattern of Microorganisms Isolated from Junk and Street Foods Sold in Akure Metropolis, Nigeria. *Jordan Journal of Biological Science*. 2016;9(4):227–234.

8. *WHO estimates of the global burden of foodborne diseases: Foodborne disease burden epidemiology reference group 2007-2015*. World health organization; 2015. 268 p.

9. *Antibiotic Resistance in Foodborne Outbreaks*. Washington DC: Center for Science in the Public Interest (CSPI); 2013.

10. Economou V, Gousia P, Kansouzidou A. Prevalence, antimicrobial resistance and relation to indicator and pathogenic microorganisms of Salmonella enterica isolated from surface waters within an agricultural landscape. *Int J Hyg Environ Health*. 2013;216(4):435–444.

11. Chen M, Love KT, Chen Y, et al. Rapid discovery of potent siRNA containing lipid nanoparticles enabled by controlled microfluidic formulation. *Journal of American Chemical Society*. 2012;134(16):6948–6951.

12. Cappuccino GJ, Sherman N. *Microbiology, A laboratory manual: Biochemical activities of microorganism*. 5th ed. California: Benjamin/Cumming science Publishing; 1999. 93–116 p.

13. Cheesbrough M. *District laboratory practice in Tropical Countries, part 2*. Cambridge University Press; 2000.

14. Krieg NR, Staley JT, Brown, DR, et al. *The Bacteroidetes, Spirochaetes, Tenericutes (Mollicutes), Acidobacteria, Fibrobacteres, Fusobacteria, Dictyoglomi, Gemmatimonadetes, Lentisphaerae, Verrucomicrobia, Chlamydiae, and Planctomycetes, second ed. (volume 4)*. New York: Springer; 2010. 211–823 p.

15. Barnett HL, Barry BH. *Illustrated genera of imperfect fungi*. Minnesota: Burgess Publishing Company; 2000.

16. Performance Standards for Antimicrobial Susceptibility Testing; Approve standard seventh ed. CLSI Document M02-A11. Pennsylvania, USA: Clinical and Laboratory Standards Institute (CLSI); 2012.

17. Nagarajan P. Rajagopalan V. Enhanced bioactivity of ZnO nanoparticles antimicrobial study. *Sci Technol Adv Mater*. 2008;9(3):035004.

18. Aluko OO, Ojeremi TT, Olaleke DA, et al. Evaluation of food safety and sanitary practices among food vendors at car parks in Ile Ife, Southwestern, Nigeria. *Food Control*. 2014;40:165–171.

19. Campos J, Gil J, Mourão J, et al. Ready-to-eat street-vended food as a potential vehicle of bacterial pathogens and antimicrobial resistance: An exploratory study in Porto region, Portugal. *Int J Food Microbiol.* 2015;206:1–6.

20. Adolf JNP, Azis BS. Microbiological status of various foods served in elementary school based on social economic status differences in Karawaci Region, Tangerang District – Indonesia. *Int Food Res J*. 2012;19(1):65–70.

21. Kim MJ, Kim SA, Kang YS, et al. Microbial diversity and prevalence of foodborne pathogens in cheap and junk foods consumed by primary schoolchildren. *Lett Appl Microbiol*. 2013;57(1):47–53.

22. Adams MR, Moss MO. *Food Microbiology*. 4th ed. New Delhi: New Age International; 2009. 205–207 p.

23. Aboloma RI. Microbiological analysis of bread samples from bakery to sale points in Ado-Ekiti, Nigeria. *Biological and Environmental Sciences Journal for the Tropics*. 2008;5(3):73–81.

24. Akinyemi KO, Fashola MO, Habib N, et al. Vended foods in Lagos, Nigeria: A potential reservoir for the spread of emerging strains of drug resistant bacteria. *Health*. 2013;5(4):675–680.

25. Sunde M, Nordstrom M. The prevalence of, associations between and conjugal transfer of antibiotic resistance genes in *Escherichia coli* isolated from Norwegian meat and meat products. *J Antimicrob Chemother*. 2006;58(4):741–747.

26. Thong KL, Modarressi S. Antimicrobial resistant genes associated with *Salmonella* from retail meats and street foods. *Food Res Int.* 2011;44(9):2641–2646.

27. Economou V, Gousia P, Kansouzidou A, et al. Prevalence, antimicrobial resistance and relation to indicator and pathogenic microorganisms of *Salmonella enterica* isolated from surface waters within an agricultural landscape. *Int J Hyg Environ Health.* 2013;216(4):435–444.

28. Wang L, Hu C, Shao L. The antimicrobial activity of nanoparticles: present situation and prospects for the future. *Int J Nanomedicine*. 2017;12:1227–1249.

29. Ramalingam B, Parandhaman T, Das SK. Antibacterial effects of biosynthesized silver nanoparticles on surface ultrastructure and nano-mechanical properties of gram-negative bacteria viz. *Escherichia coli and Pseudomonas aeruginosa*. ACS Appl Mater Interfaces. 2016;8(7):4963–4976.

30. Soni H, Kumar NJI, Patel K, et al. Metallic nanoparticles as alternative antimicrobial. *Journal of Nanomedicine Research*. 2017;6(4):00165.

31. Shahid S, Khan SA, Ahmad W, et al. Size-dependent bacterial growth inhibition and antimicrobial activity of Ag-doped ZnO nanoparticles under different atmospheric conditions. *Indian Journal of Pharmaceutical Sciences*. 2018;155(1):173–180.

32. Gurunathan S, Han JW, Dayem AA, et al. Oxidative stress-mediated antibacterial activity of graphene oxide and reduced graphene oxide in *Pseudomonas aeruginosa*. *Int J Nanomedicine*. 2012;7:5901–5914.

33. Nagy A, Harrison A, Sabbani S. et al. Silver nanoparticles embedded in zeolite membranes: release of silver ions and mechanism of antibacterial action. *Int J Nanomedicine*. 2011;6:1833–1852.

34. Leung YH, Ng AM, Xu X, et al. Mechanisms of antibacterial activity of MgO: non-ROS mediated toxicity of MgO nanoparticles towards *Escherichia coli*. *Small*. 2014;10(6):1171–1183.