PREVALENCE AND ANTIBIOTIC RESISTANCE PROFILE OF STAPHYLOCOCCUS AUREUS ISOLATED FROM FRESH FRUITS AND VEGETABLES SOLD IN BOKKOS CENTRAL MARKET, JOS PLATEAU STATE NIGERIA

Musa Filibus Gugu[1]*, Solomon Naku Bazing Magaji[2], Samira Aliyu Muhammad[2], Diyak Enoch Mimang[1]

[1]*Department of Microbiology, Faculty of Natural and Applied Sciences, Plateau State University, P. O. Box 2012, Bokkos, Nigeria*
[2]*Department of Biomedical Engineering Technology, Institute of Allied Health Sciences and Technology, Kaduna State University, P.M.B. 1050 Makarfi Kaduna State, Nigeria*
***Correspondence:** mfgugu@plasu.edu.ng*

ABSTRACT

There is a remarkable increase in the burden of food-borne diseases because of antimicrobial resistance which poses a greater risk to effective treatment. However, very little is known about the antibiotic resistance profile of microorganisms isolated from fruits and vegetables sold in Bokkos market in Plateau State, Nigeria. This study was conducted to examine the antibiotic resistance profile of Staphylococcus aureus Isolated from fresh fruits and vegetables sold in Bokkos market. A total of 80 fresh fruits and vegetable samples were collected from eight (8) different fruits and vegetables in the market and analysed. The fruits and vegetables analysed are Pepper (P), Tomato (T), Garden egg (G), Carrot (Ca), Cucumber (Cu), Watermelon (M), Sweet orange (O), and Banana (B). The prevalence of isolated S. aureus was higher in carrots and cucumber (16% each), while it was lower in watermelon (10%), oranges (10%) and tomatoes (8%). The antibiotics used with their concentrations are Ampiclox (AMP 20µg), Ciprofloxacin (CPX 10µg), Chloramphenicol (CH 30µg), Gentamycin (CN 10µg), Norfloxacin (NB 10µg), Streptomycin (S 30µg), Rifampicin (R 20 µg), Erythromycin (E 30µg), Levofloxacin (L 20µg), and Amoxil (AML 20µg). S. aureus isolates from carrot, watermelon and garden egg were resistant to all the antibiotics, while isolates from tomatoes only were susceptible to all the antibiotics. The presence of antibiotic resistant S. aureus in fresh fruits and vegetables in Bokkos market of Plateau State Nigeria suggests a great threat to public health and safety for the people of Bokkos local government

Keywords: *Antibiotic, Staphylococcus aureus, Resistance, Fruits and vegetables, Bokkos market*

1. INTRODUCTION

An antibiotic is a substance produced by one microorganism which in low concentration can kill or inhibit the growth of another organism [1]. There has been an increase in the resistance of bacteria isolated from food sources to common antimicrobial agents over time [1]. Consumers have become alarmed over the presence of resistant microorganisms to antibiotics on food products [1]. Reports have it that the level of resistance to antibiotics has been on the increase among food-borne pathogens such as *Staphylococcus, Salmonella and Shigella* [2].

Fruits and vegetable are predisposed to contamination by potentially pathogenic microorganisms on the field and consumption of such fresh fruits poses a great risk of food borne diseases. There has been a noticeably higher food borne disease outbreaks in the past decades associated with the consumption of fresh fruits and vegetables [3]. Fruits and vegetable crops were the most implicated items for several food-borne disease outbreaks spanning 1973-2014 [4]. Soil, contaminated irrigation water, improper human handling of produce and inadequately composted manure are the common sources of pre- and post-harvest contaminations [5].

Staphylococcus aureus is a pathogenic bacterium which causes food borne diseases along with several other diseases. It is also the causative agent of intoxication and gastroenteritis [6]. *Staphylococcus aureus* is a ubiquitous, versatile, and highly adaptive pathogen that colonizes the skin and mucous membrane of the anterior nares, gastrointestinal tracts, perineum, the genitourinary tracts, and pharynx [7]. It is a host specialized organism with the ability to gain and lose resistance and virulence genes. It is an organism of public health importance due to its zoonotic potential [8].

S. aureus is clinically the most pathogenic member of the genus *Staphylococcus* having a wide etiological implication in a range of diseases such as superficial skin abscess, food poisoning and life-threatening diseases such as bacteremia, necrotic pneumonia in children and endocarditis [9]. It causes septicemia and arthritis in poultry, dermatitis in dogs, mastitis in cows, and botryomycosis in horses [8]. The possession of antibiotic-resistant genes and the production of several putative virulence factors in Staphylococcus makes its disease very severe. The Antibiotic resistant genes: mecA, VanA, Staphylococcus exotoxins and other factors facilitate the initiation of the disease process coupled with its ability to cause host tissue destruction and evasion of host immunity [9].

Resistance to antibiotics by *Staphylococcus aureus* is developed through different means, such as horizontal gene transfer of different mobile genetic elements (MGEs), including bacteriophages, plasmids, Staphylococcus cassette chromosomes (SCCs), transposons and pathogenicity islands (PAIs). The MGEs mentioned above are potential carriers of antibiotic-resistant genes and can be predicted by the size of the plasmid. Small plasmids may possess resistant genes to chloramphenicol, tetracycline, and erythromycin, whereas resistance against beta-lactams, macrolides and aminoglycosides is carried by large plasmids. Larger plasmids on the other hand carry resistant genes to beta-lactams, spectinomycin, erythromycin, vancomycin, and trimethoprim when they integrate with other MGEs [10]. To date, there is no documented report on antibiotic resistance of *S. aureus* in plant or plant products in Bokkos Local government area of Plateau State, Nigeria. Hence, this research was conducted to provide documented evidence on the antibiotic resistance of *S. aureus* in fresh fruits and vegetables within the study area.

MATERIALS AND METHODS

Sample collection

A total of eighty (80) samples, ten (10) each from eight different fruits were aseptically collected into different sterile polythene bags, labelled, and transported in a cold chain to the Microbiology laboratory at National Veterinary Research Institute (NVRI), Vom for analyses. The fruit and vegetable samples collected are listed thus: Pepper (P), Tomato (T), Garden egg (G), Carrot (Ca), Cucumber (Cu), Watermelon (M), Sweet orange (O), and Banana (B).

Bacteria isolation

The organism was isolated from fruits and vegetables according to the methods of Effiuvwevwere and Faiers [11, 12]. A sterile blade was used to slice a portion, approximately 1g and aseptically inoculated into peptone water and incubated at 37ºC for 24 hours. A portion of the overnight broth was streaked onto Mannitol Salt Agar (MSA) and Nutrient Agar (NA) using a sterile wire loop and incubated at 37ºC for 24 hours [12].

Colonies were distinguished on the bases of cultural characteristics such as colony size, shape, colour, consistency and haemolytic characteristics [13]. The bacterial growth was sub-cultured onto MSA and onto NA slant and incubated at 37ºC for 24 hours. Gram staining, microscopy, and different biochemical tests such as coagulase, catalase, Oxidase, glucose, maltose, sucrose, Mannitol, lactose, urease, and indole were performed to confirm the presence of *S. aureus*. The organism was morphologically compared with bacteria identification atlas for further confirmation [14].

Antibiotic sensitivity test of *Staphylococcus aureus*

The antibiotic resistance of *Staphylococcus aureus* isolated from fruits and vegetables was determined on Nutrient Agar using Kirby-Bauer disk diffusion method [15]. A little loop of the sample was taken using a wire loop and streaked all over the NA. The antibiotic disk was aseptically placed on the surface of the NA plate, it was allowed to absorb for few minutes and then incubated at 37ºC for 24 hours. The presence of zones of inhibition around the antibiotic disk indicates microbial inhibition and was measured to the nearest millimetre using a well calibrated meter ruler and recorded.

The antibiotics used were Ampiclox (AMP 20µg), Ciprofloxacin (CPX 10µg), Chloramphenicol (CH 30µg), Gentamycin (CN 10µg), Norfloxacin (NB 10µg), Streptomycin (S 30µg), Rifampicin (R 20 µg), Erythromycin (E 30µg), Levofloxacin (L 20µg), Amoxil (AML 20µg). All disks were manufactured by OPTUN LABORATORIES NIG. LTD

Statistical Analysis

Data obtained was analysed using R Console version 4.0.2. The susceptibility numbers were converted to base-10 logarithms for statistical analysis. The difference in the susceptibility rate of different antibiotics on *S. aureus* was compared using Pearson's Chi-square test. The level of significance was set at $P < 0.05$.

RESULTS

The biochemical characteristics of *Staphylococcus aureus* isolated from fresh fruits and vegetables are shown in Table 1. From the table, only catalase and coagulase tests were positive for *S. aureus* isolated from all samples

Table 1. Biochemical characteristics of *Staphylococcus aureus* isolated from fresh fruits and vegetables

	Sample Types							
	O	Ca	Cu	B	P	M	T	G
Biochemical test								
Catalase	+	+	+	+	+	+	+	+
Coagulase	+	+	+	+	+	+	+	+
Oxidase	-	-	-	-	-	-	-	-
Glucose	+	-	-	+	-	+	-	-
Maltose	-	-	+	-	-	+	-	-
Sucrose	-	-	-	+	-	-	-	-
Mannitol	-	+	-	-	+	-	-	-
Lactose	-	-	-	-	-	-	+	-
Urease	-	-	-	-	-	-	-	-
Indole	-	-	-	-	-	-	-	-

Key: + = **positive,**
- = **negative**

The percentage positive of *Staphylococcus aureus* isolated from each sample type is indicated in Figure 1. Their percentages shows that Cucumber and carrot have the highest percentage of occurrence (16%), while tomatoes have the least (8%).

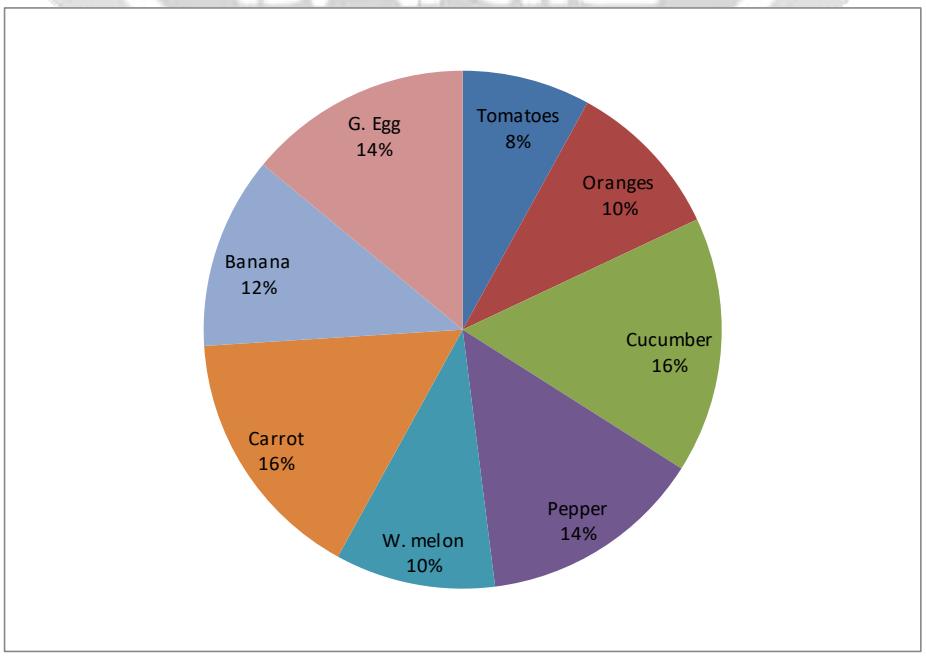

Figure 1. Percentage positive of *Staphylococcus aureus* isolated from fresh fruits and Vegetables

The antibiotic susceptibility test of each isolate, taken from each sample type are shown in Figure 2. The entire selected antibiotics were resistant to at least one antibiotic, except in tomato where the isolated organism was susceptible to all the antibiotics. *S. aureus* isolated from Watermelon, Garden egg, and Carrot were all resistant to the selected antibiotics used. Streptomycin (S 30µg) shows the maximum zone of inhibition in oranges (27mm), while Gentamycin (CN 10µg) shows the minimum zone of inhibition (12mm) on cucumber (Figure 2).

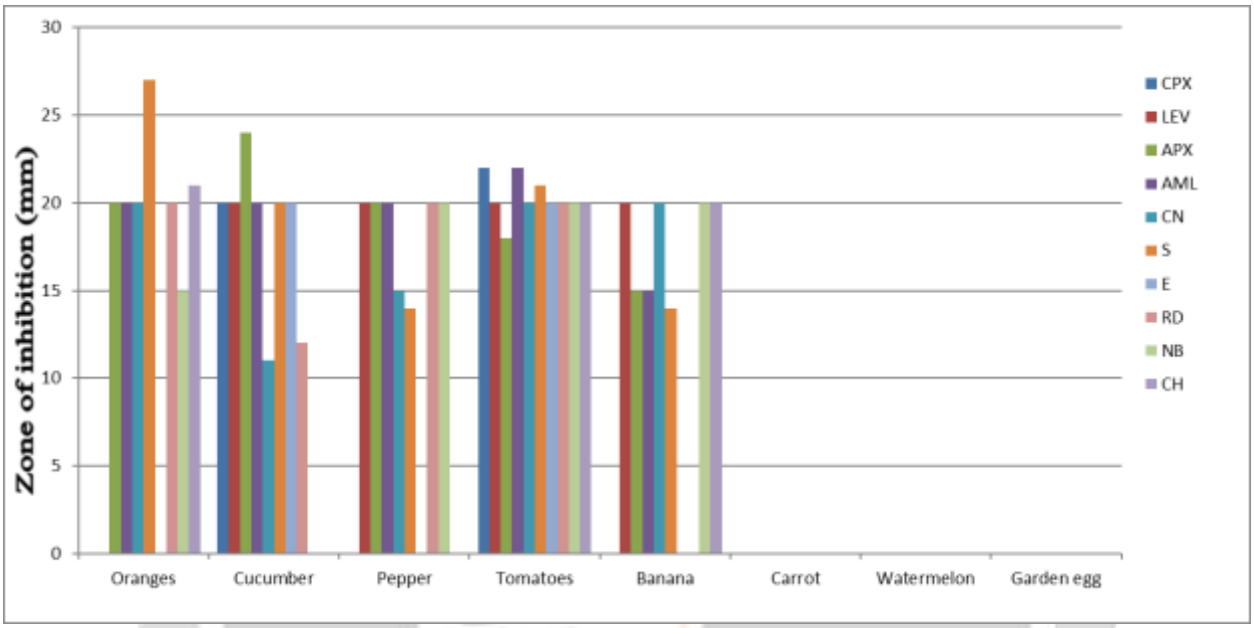

Figure 2. Antibiotic susceptibility test of S. aureus recovered from fresh fruits and vegetables [in diameter (mm)]

Key: CPX, Ciproflox; NB, Norfloxacin; AML, Amoxil; CN, Gentamycin; S, Streptomycin; RD, Rifampicin; E, Erythromycin; CH, Chloramphenicol; APX, Ampiclox; LEV, Levofloxacin

The antibiotic resistance profile of preselected resistant isolates recovered from fresh fruits and vegetables is shown in Table 2. The entire selected antibiotics were resistant to at least one antibiotic used in each sample type, except in tomatoes that were all susceptible to the antibiotics used (Table 2). From the table, the order of resistance of the isolates is carrot, watermelon, garden egg, oranges, pepper, banana, and cucumber which showed the least number of resistant isolates. The table also shows each sample type with its resistance profile

Table 2. Antibiotic resistance profile of isolates recovered from fresh fruits and vegetables.

S/N	SAMPLE TYPE	RESISTANCE PROFILE
1	Oranges	CPX, E, LEV
2	Cucumber	NB, CH
3	Carrot	CPX, NB, AML, CN, S, RD, E, CH, APX, LEV
4	Pepper	CPX, E, CH
5	Tomatoes	ALL SENSITIVE TO THE TESTED ANTIBIOTICS
6	Watermelon	CPX, NB, AML, CN, S, RD, E, CH, APX, LEV
7	Banana	CPX, RD, E.
8	Garden egg	CPX, NB, AML, CN, S, RD, E, CH, APX, LEV

Key: CPX,9u Ciproflox; NB, Norfloxacin; AML, Amoxil; CN, Gentamycin; S, Streptomycin; RD, Rifampicin; E, Erythromycin; CH, Chloramphenicol; APX, Ampiclox; LEV, Levofloxacin.

DISCUSSION

The presence of *S. aureus* in fruits and vegetables in Bokkos local government area of Plateau has not been documented. In this study, we looked at some fruits and vegetables in Bokkos Local Government of Plateau State Nigeria, for the presence of *S. aureus* and its resistance to different antibiotics. *S. aureus* was detected in all the fruits and vegetable samples examined. The prevalence of *S. aureus* varies significantly among samples ($P < 0.05$). *S. aureus* was higher in cucumber and carrot (16%), and least in Oranges (10%), watermelon (10%) and tomatoes (8%). This result coincides with a similar study conducted on carrots in Zaria Nigeria, where the prevalence of isolated *S. aureus* was higher than that of isolated *E. coli* on the same fruit [16]. In another study conducted in Korea on fresh fruits and vegetables involving lettuce, mixed salads and green onions, *S. aureus* was implicated in the contamination of the fruits and vegetables [17]. The same organism was also isolated from radish, lettuce, and seed sprouts [18]. Vegetables can be contaminated with pathogens during growth, harvest, post-harvest handling and distribution [19]. This could be a result of unsuitable human management and storage, infected irrigation water, containers, animal waste fertilisers and post-harvest washings [20].

Since *S. aureus* can be resistant to some antimicrobials, this study investigated the resistance pattern of the organism to some of these antimicrobials as shown in figure 2. The organisms isolated from carrot, watermelon and garden egg were completely resistant to all the antibiotics; ciproflox, norfloxacin, amoxil, gentamycin, streptomycin, rifampicin, erythromycin, chloramphenicol, ampiclox, levofloxacin. This result also coincides with a similar study in Korea where most *S. aureus* isolates from leafy vegetables showed resistance to several antibiotics [21]. All *S. aureus* isolates from tomatoes were sensitive to all the antibiotics. This conforms with a study on retail vegetables in China [22].

This high resistance of *S. aureus* observed in some of the antibiotics may be because of the general use of animal manure as fertilisers in vegetable farming. Tetracycline, sulfonamides, and quinolone antibiotics had been detected in animal manures (chicken, livestock, and swine) from China [23]. Several other factors play a key role in increasing the burden of environmental resistant bacteria

These factors include human activities such as emissions of antibiotic residues, fish production, animal production, wastewater treatment, and antibiotic manufacturing. In addition, antibiotics may be taken up by vegetables and fruits planted in the soil contaminated by antibiotics [24].

CONCLUSION AND RECOMMENDATION

In summary, the presence of *S. aureus* in fruits and vegetables sold in Bokkos markets is worrying. This shows that fruits and vegetables eaten raw are a likely threat of foodborne bacterial infection. Increased antibiotic resistance among *S. aureus* isolated from fruits and vegetables obtained in most markets is a first-rate health issue to the society. The findings in this work highlights the need for strict hygiene requirements in the sale of fruits and vegetables in Bokkos markets to reduce the prevalence of *S. aureus*. The use of handled animal manure in agricultural practices needs proper attention to reduce the spread of antimicrobial resistant microorganisms in fruits and vegetables. This work, therefore, highlights the danger associated with fresh fruits and vegetables directly from Bokkos market

REFERENCES

1. Visca, M. S. & Singh, N. P. (2010). Multidrug resistant Acinetobacter. *Journal of global infectious disease.* 2:291-304

2. Mache, A. (2002). Salmonella serogroups and their antibiotic resistance patterns isolated from diarrhea stools of pediatric outpatients in Jimma hospitals and Jimma health center, southwest Ethiopia. *Ethiopia journal of health science*. 12:37-45
3. Humphreys, H. (2012). *Staphylococcus aureus*: the enduring pathogen in surgery. *Surgeon* 10, 357–360.

4. Herman, K. M., Hall, A. J., & Gould, L. H. (2015). Outbreaks attributed to fresh leafy vegetables, United States, 1973-2012. *Epidemiology and Infection*,143(14), 3011–3021

5. Tomas-Callejas, A., Lopez-Velasco, G., Camacho, A. B., Artes, F., Artes-Hernandez, F., & Suslow, T. V. (2011). Survival and distribution of *Escherichia coli* on diverse fresh-cut baby leafy greens under preharvest through postharvest conditions. *International Journal of Food Microbiology*, 151(2), 216–222

6. Tong, S.Y., Davis, J.S., Eichenberger, E., Holland, T. L. & Fowler, V. G. (2015). *Staphylococcus aureus* infections: epidemiology, pathophysiology, clinical manifestations, and management. *Clinical Microbiology Reviews*. 28 (3): 603–61

7. Sakr, A., Brégeon, F., Mège, J. L., Rolain, J. M. & Blin, O. (2018). *Staphylococcus aureus* Nasal Colonization: An Update on Mechanisms, Epidemiology, Risk Factors, and Subsequent Infections. *Frontiers in Microbiology* 8;9:2419.

8. Luzzago, C., Locatelli, C., Franco, A., Scaccabarozzi, L., Gualdi, V., Viganò, R., Sironi, G., Besozzi, M., Castiglioni, B., Lanfranchi, P. & Cremonesi, P. (2014). Clonal diversity, virulence-associated genes and antimicrobial resistance profile of *Staphylococcus aureus* isolates from nasal cavities and soft tissue infections in wild ruminants in Italian Alps. *Veterinary Microbiology*, 170(1):157-61.

9. Shaw, L., Golonka, E., Potempa, J. and Foster, S.J. (2004). The role and regulation of the extracellular proteases of Staphylococcus aureus. *Microbiology*, 150(1): 217-228.

10. Bitrus, A. A., Peter, O. M., Abbas, M. A., Goni, M. D. (2018). *Staphylococcus aureus*: A Review of Antimicrobial Resistance Mechanisms. *Veterinary Sciences: Research and Reviews*. 2018;4(2).

11. Effiuvwevwere, B. J. O. (2000). Microbial Spoilage Agents of Tropical and Assorted Fruits and Vegetables: An Illustrative Reference Book. Paragraphics Publishing Company, Port Harcourt, Nigeria, pp: 1-39.

12. Faiers, M., R., George, J. J., & Wheat, P. (1991). *Multipoint Methods in Clinical Laboratory*. 3rd Edn., University Press Cambridge, Great Britain, pp: 21-28

13. Fawole, M.O. & Oso, B. A. (1995). *Laboratory Manual of Microbiology*. Spectrum Books Limited, Ibadan, Owerri, Kaduna and Lagos, pp: 71-81

14. Cullimore, D. R. (2010). *Practical Atlas for Bacterial Identification*. 2nd ed. CRC Press

15. Jorgensen, J. H., & Turnidge, J. D. (2007). Susceptibility test methods: dilution and disk diffusion methods, p. 1152–1172. In P. R. Murray, E. J. Baron, J. H. Jorgensen, M. L. Landry, and M. A. Pfaller (ed.), *Manual of clinical microbiology*, 9th ed. ASM Press, Washington, D.C.

16. Bishop, H. G. & Okwori, G. O. (2017). *Escherichia coli* and *Staphylococcus aureus* contaminations of carrots sold within Zaria, Nigeria and their antibiotic susceptibility profiles. *Journal of Science*.;1(4):110–113.

17. Seo, Y. H., Jang, J. H. & Moon, K. D. (2010). Occurrence and characterisation of enterotoxigenic *Staphylococcus aureus* isolated from minimally processed vegetables and sprouts in Korea. *Food Science and Biotechnology* 19(2), 313-319.

18. Nguz, K., Shindano, J., Samapundo, S. & Huyghebaert, A. (2005). Microbiological evaluation of fresh-cut organic vegetables produced in Zambia. *Food Control* 16(7), 623-628

19. Olaimat, A. N., Delaquis, P. J. & Holley, R. A. (2016). Impact of materials handling at pre-and post-harvest operations on the microbial ecology of foods of vegetable origin. *Quantitative Microbiology in Food Processing*: *Modeling the Microbial Ecology* 2016, 85- 116.

20. Mritunjay, S. K. & Kumar, V. (2015). The fresh farm produces as a source of pathogens: A review. *Research Journal of Environmental Toxicology* 9(2), 59-70

21. Hong, J., Kim, Y., Kim, J., Heu, S., Kim, S. R., Kim, K. P. & Roh, E. (2015). Genetic diversity and antibiotic resistance patterns of *Staphylococcus aureus* isolated from leaf vegetables in Korea. *Journal of food science* 80(7), 1526-1531.

22. Wu, S., Huang, J., Wu, Q., Zhang, F., Zhang, J., Lei, T., Chen, M., Ding, Y. & Xue, L. (2018) Prevalence and Characterization of *Staphylococcus aureus* isolated from retail vegetables in China. *Frontiers in Microbiology*. 9:1263

23. Gao, J., Ferreri, M., Yu, F., Liu, X., Chen, L., Su, J. & Han, B. (2012). Molecular types and antibiotic resistance of *Staphylococcus aureus* isolates from bovine mastitis in a single herd in China. *The Veterinary Journal* 192(3), 550-552

24. Hu, X., Zhou, Q. & Luo, Y. (2010). Occurrence and source analysis of typical veterinary antibiotics in manure, soil, vegetables and groundwater from organic vegetable bases, northern China. *Environmental Pollution* 158(9), 2992-2998