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The Role of Laboratory Professionals in Preventing Contamination of Food by Antimicrobial-Resistant Microorganisms (AMRMs)

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ABSTRACT

Antimicrobial-resistant-microorganism (AMRM)-infected or contaminated food that leads to livestock losses and disease/death in humans is estimated to put 31 million people at risk of food insecurity in Nigeria by the last quarter of 2024. This study aimed to explicate the roles of food-industry stakeholders/laboratorians in preventing food contamination and animal/human infection by AMRMs. The objectives were to determine what food/food system, food security, and stakeholders in the food industry are; investigate the occurrence of AMRM in food; and elucidate preventive measures to be implemented to avoid AMRM in food. A structured questionnaire was administered to stakeholders and baseline information from published literature/established international standards was used to achieve this purpose. The majority (88%) of the respondents were from the Veterinary Teaching Hospital, University of Ilorin; all (100%) participants from the laboratories stated they knew the meaning of food security, while 56% of respondents that were ≤25 years old stated that AMRM could contaminate food. Only 52% of university-educated respondents agreed they were stakeholders in the food industry while 60% were veterinarians/medical practitioners. Reported contamination of food by AMRMs from harvest to consumption, especially in low/middle-income countries (LMICs) like Nigeria was by antimicrobial-resistant Staphylococcus spp., *Bacillus* spp., *Proteus* spp., *Salmonella* spp., and *Clostridium* spp. This was largely enhanced by the unrestricted use of antimicrobial agents in food animals, especially as growth promoters, leading to the production of unwholesome food, outbreaks of zoonoses, loss of livestock, and human diseases that were foodborne. Compulsory antimicrobial susceptibility tests should be done for isolates from food/food products to determine their suitability for use and the presence of AMRM. The diffusion method of antimicrobial susceptibility testing (AST) should strictly follow the International Standard Organization (ISO) protocol, which includes using pure colonies, overnight culture of 0.5 McFarland turbidity, and interpreting zones of inhibition using the Clinical and Laboratory Standard Institute (CLSI) recommended breakpoints. Only certified safe and wholesome food/food products should be passed to the public for consumption. Universities and researchers should carry out more extension work outside establishments' walls to disseminate research results in food-AMRM.

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INTRODUCTION

Antimicrobial-resistant microorganisms (AMRMs) are bacteria, viruses, parasites and fungi that change when indiscriminately exposed to antimicrobials such as antibiotics, antivirals, antiparasitics and antifungals as a result of which the antimicrobial agents become ineffective resulting in infections persisting in infected individuals thereby increasing the risk of disease spread to others (**WHO, 2021**). Antimicrobial resistance is developed through different mechanisms such as degradation of antimicrobial agents by microbes' enzymes, modification of antimicrobial targets, alteration of microbial cell wall permeability, and activation of alternate pathways (**Samtiya *et al.*, 2022**).The risk of antimicrobial resistance (AMR) due to the abuse of antimicrobial agents and limited development of new antimicrobials, coupled with their adverse effect on human/animal populations is bringing the world back to the time when microbial infections posed significant threats (**Almansour *et al.*, 2023**).

Antimicrobial-resistant organisms are found in humans, animals, and the environment, including air, soil, and water, including food. They can spread from food of animal origin to humans, person to person and between humans and animals (**Founou *et al*., 2016; Jans *et al*., 2018**). The spread of antimicrobial-resistant microorganisms (AMRMs) could be encouraged by inappropriate food handling, inadequate sanitary conditions and poor infection control (**Cheng, 2022).**

Food is any tasty object of plant and animal origin that is edible and can feed an individual **(Borghini and Piras, 2020).** It is made up of nutrients like carbohydrates, proteins, minerals, fats, and vitamins and is consumed to supply support for the body in terms of nutrition **(Khan *et al*., 2019).** Food systems are combinations of socio-economic, political, and environmental systems **(WEF, 2023).** They encompass all stakeholders and their contributory activities in achieving production, storage, distribution, processing, consumption, and disposal of food products from agriculture, fisheries, and forestry (**FAO, 2018a**). Food is one of the necessities of life **(UN, 2023).**

Food security is always having both economic and physical access to safe, sufficient, and nutritious food to meet food preferences and dietary needs for a productive, healthy, and active life **(FAO, 2006).** This is attained if the population does not live in hunger or fear of hunger **(USAID, 2023).** Globally, since 2020, hunger has been on the rise again (**NPA, 2023**). In 2021, as many as 2.3 billion people, which is 29.3% of the world's population faced moderate or severe food insecurity. This was reported to be an increase of 350 million people compared to 2020 **(UNN, 2022).**

Food insecurity has been attributed to varying factors including poverty, drought, conflict, inflation, climate change, rising food prices, unhealthy eating habits, and consumption of unwholesome food **(Smith *et al*., 2022; UNN, 2022; UNICEF, 2023).** The food security and nutrition situation across Nigeria is reported to be deeply concerning. Over 25 million people were projected to be food insecure by August 2023 **(UNICEF, 2023).** A stakeholder describes any person or group of persons who have an interest or stake in an issue. He is affected by or can affect the issue or situation and the organization's objectives achievement **(FAO, 2018b).** From food security's point of view, a distinction needs to be made between citizens who are food-security-right-holders but also the most vulnerable to food insecurity and the duty-bearer, who is mainly the government that should fulfill people's right to adequate food **(Beals, 2023).**

Stakeholders in the food industry are extensive and complexly interrelated **(Thiry, 2021; UNEP-FAO-UNDP, 2023).** They include farmers, farm input suppliers, food producers/manufacturers, processors, and packagers **(EFSA, 2023).** Others include individual/corporate consumers, vendors, retailers, distributors, and market managers. Private sector stakeholders include food produce transporters, hotels, restaurants, and caterers **(GAFIN, 2020).** Professional associations, public health groups, non-governmental organizations (NGOs), and international organizations like the Food and Agricultural Organization (FAO) have a vested interest in the food industry **(Beals, 2023).** The importance of research and the academic sector in the food industry cannot be overemphasized; this includes research institutes/organizations and universities **(EFSA, 2023).** Governments at all levels including ministries, departments, and agencies (MDAs), have an obligatory stake in the food industry **(GAFIN, 2020).** Other stakeholders in the food industry include women's groups, indigenous people, children, youth, workers, and trade unions involved in the food production chain **(EFSA, 2023).**

Stakeholders in the food industry, therefore, have important roles to play in addressing the threats emanating from AMRM contamination in food to save all species on the planet from food insecurity and the catastrophic public health crisis of AMR (**Nambiar and Asha, 2023**). This study aimed to explicate the roles of food-industry stakeholders/laboratory professionals in preventing food contamination by AMRMs.

MATERIALS AND METHODS

Sample and populations of the study

This study was carried out within the Ilorin South Local Government Areas of Ilorin metropolis. Ilorin is the capital of Kwara State, located in the North Central region of Nigeria. The city is located at latitude 8.500^0 N and longitude 4.550^0 E, it is 765 km² in area and situated about 320 m above sea level. It is strategically a gateway between the Northern and Southern parts of the country (**Aiyedun *et al*., 2017**). To the best of our knowledge, there is a paucity of published work in the area of research of this study. Fifty percent (25) of the 49 (VPH&PM Lab-2; VTH Lab-43; Aromokeye Lab-2; and Fleming Lab-2) identified laboratory practitioners in the study area were selected randomly and participated in this study's survey (**Table 1**).

Source of data collection and instruments

A structured questionnaire **(table 1)** was administered to 25 stakeholders from laboratories in the transit city of Ilorin, Nigeria to collect data concerning food security, their stake in the food industry, the possibility of AMRM contaminating food, and if it could be avoided including the possibility of contamination being avoided by the observance of antimicrobial susceptibility testing (AST) standards.

Additionally, baseline information from published literature was also sourced using the Google

search engine. The keywords that were used for the search were food security; stakeholders in the food industry; antimicrobial-resistant microorganisms (AMRMs); contaminated food; laboratorians and antimicrobial sensitivity testing standards. Relevant publications were saved and retrieved later for use in the study.

Established international standards from the International Standard Organization (ISO), Clinical Laboratory Standard Institute (CLSI), and World Health Organization (WHO) were also used to achieve the purpose of this study.

Methods of data analysis

Analyses and presentation of obtained data were done using Microsoft Word, Microsoft Excel, tables, and other quantitative representations.

Table 1: A structured questionnaire

SECTION A: Demographic Profile

1. Gender: Male ☐ Female ☐
2. Age (years) ≤25 ☐ >25 ☐
3. Educational level
   - None ☐
   - Primary Sch ☐
   - Secondary Sch ☐
   - University ☐
4. Job designation:
   - Lab. Assistant ☐
   - Lab. Technologist ☐
   - Veterinarian/Medical Practitioner ☐
   - Others ☐

SECTION B: The Role of Laboratory Professionals in Preventing Contamination of Food by Antimicrobial-Resistant Microorganisms (AMRMs)

1. Do you know the meaning of food security? Yes ☐ No ☐
2. Are you a stakeholder in the food industry? Yes ☐ No ☐
3. Can food be contaminated by antimicrobial resistant microorganisms? Yes ☐ No ☐
4. Can contamination of food by antimicrobial resistant microorganisms be avoided? Yes ☐ No ☐
5. Can the observance of antimicrobial sensitivity test standards aid in avoiding food contamination by antimicrobial-resistant microorganisms? Yes ☐ No ☐

RESULTS

The majority (88%) of the respondents were from the Veterinary Teaching Hospital, Ilorin, while 68%, 100%, and 60% were males, university graduates, and veterinarians/medical practitioners respectively **(Table 2).**

Table 2: Demographic Data for Respondents of The Role of Laboratory Professionals in Preventing Contamination of Food by Antimicrobial-Resistant Microorganisms (AMRMs)

S/N	Laboratory	No (%)	Gender	No (%)	Age	No (%)	Educational Level	No (%)	Job description	No (%)
1	VPH&PM Lab	1 (4)	Male	17 (68)	>25	11 (44)	None	0 (0)	Lab. Assistant	6 (24)
2	VTH Lab	22 (88)	Female	8 (32)	≤25	14 (56)	Primary Sch	0 (0)	Lab. Technologist	2 (8)
3	Aromokeye Lab	1 (4)					Secondary Sch	0 (0)	Veterinarian/ Medical Practitioner	15 (60)
4	Fleming Lab	1 (4)					University	25 (100)	Others	2 (8)
	Total	25(100)		25(100)		25(100)		25(100)		25(100)

All (100%) of the respondents from the laboratories stated they know the meaning of food security while 96% of the respondents stated that AMRM can contaminate food and that contamination of food by AMRM can be avoided by the observance of AST standards **(Table 3).** More (68%) male respondents stated they knew the meaning of food security compared to female respondents, 36% agreed they were stakeholders in the food industry, and 68% stated that AMRM can contaminate food while 64% stated that contamination of food by AMRM can be avoided by the observance of AST standards **(Table 3).**

The proportion of respondents that were ≤25 years old who stated that AMRM can contaminate food and that contamination could be avoided by observance of AST standards was 56% each. Only 52% of the respondents with university education agreed they were stakeholders in the food industry compared to 48% that stated otherwise, while 96% of them opined that AMRM can contaminate food and that food contamination can be avoided by the observance of AST standards. Among the respondents, veterinary/medical practitioners and those that stated that food can be contaminated by AMRM were sixty percent (60%) each. Fifty-six percent (56%) and 60% of the respondents stated that food contamination can be avoided and that the contamination could be avoided through the observance of AST standards, respectively **(Table 3).**

Table 3: Response from Respondents of The Role of Laboratory Professionals in Preventing Contamination of Food by Antimicrobial-Resistant Microorganisms (AMRMs)

S/N	Variables	Do you know the meaning of food security?		Are you a stakeholder in the food industry?		Can antimicrobial-resistant microorganisms contaminate food?		Can contamination of food by antimicrobial-resistant microorganisms be		Can the observance of antimicrobial sensitivity test standards aid in avoiding food	
		Yes (%)	No (%)	Yes (%)	No (%)	Yes (%)	No (%)	Yes (%)	No (%)	Yes (%)	No (%)
1	**Laboratory**										
	VPH&PM Lab	1(4)	0(0)	1(4)	0(0)	1(4)	0(0)	1(4)	0(0)	1(4)	0(0)
	VTH Lab	22(88)	0(0)	11(44)	11(44)	22(88)	0(0)	21(84)	1(4)	21(84)	1(4)
	Aromokeye Lab	1(4)	0(0)	0(0)	1(4)	0(0)	1(4)	1(4)	0(0)	0(0)	1(4)
	Flemng Lab	1(4)	0(0)	1(4)	0(0)	1(4)	0(0)	1(0)	0(0)	1(4)	0(0)
2	**Gender**										
	Male	17(68)	0(0)	9(36)	8(32)	17(68)	1(4)	16(64)	1(4)	16(64)	1(4)
	Female	8(32)	0(0)	4(16)	4(16)	7(28)	0(0)	8(32)	0(0)	8(32)	0(0)
3	**Age**										
	>25	11(44)	0(0)	8(32)	3(12)	10(40)	1(4)	10(10)	1(4)	10(40)	1(4)
	≤25	14(56)	0(0)	5(20)	9(36)	14(56)	0(0)	14(56)	0(0)	14(56)	0(0)
4	**Educational Level**										
	None	0	0	0	0	0	0	0	0	0	0
	Primary Sch	0	0	0	0	0	0	0	0	0	0
	Secondary Sch	0	0	0	0	0	0	0	0	0	0
	University	25(100)	0(0)	13(52)	12(48)	24(96)	1(4)	24(96)	1(0)	24(96)	1(4)
5	**Job description**										
	Lab. Assistant	6(24)	0(0)	1(4)	5(20)	6(24)	0(0)	6(24)	0(0)	6(24)	0(0)
	Lab. Technologist	2(8)	0(0)	0(0)	2(8)	1(4)	1(4)	2(8)	0(0)	1(4)	1(4)
	Veterinarian/Medical Practitioner	15(60)	0(0)	10(40)	5(20)	15(60)	0(0)	14(56)	1(4)	15(60)	0(0)
	Others	2(8)	0(0)	2(8)	0(0)	2(8)	0(0)	2(8)	0(0)	2(8)	0(0)
6	**General result**	25(100)	0(0)	13(52)	12(42)	24(96)	1(4)	24(96)	1(4)	23(92)	2(8)

VPH&PM Lab- Veterinary Public Health &Preventive Medicine Laboratory, VTH Lab- Veterinary Teaching Hospital Laboratory, Lab- Laboratory, Sch- School

Many published works and the World Health Organization have reported the occurrence of microorganisms and antimicrobial-resistant organisms in food in Nigeria. These included *Staphylococcus saprophyticus* (20%), *Proteus vulgaris* (10%), *Bacillus subtilis* (10%), *Proteus mirabilis* (10%), *Micrococcus varians* (10%), *Bacillus licheniformis* (10%), *Bacillus cereus* (10%), *Bacillus polymyxa* (10%), and *Micrococcus luteus* (10%) reported by Okanlawon *et al*. from commercial rice sold in canteens within Obafemi Awolowo University (OAU), Ile-Ife. The isolation of 66.7% and 13.3%, respectively, of *Salmonella* spp. from water used in the fish and chicken processing units of the main market and abattoir in Sokoto State, Nigeria, by Faleke *et al*. Spore-forming bacteria (including *Bacillus cereus, Clostridium perfrigens*, and *Clostridium botulinum*) and non-spore-forming bacteria (*E. coli, Staphylococcus aureus, Salmonella spp.) are* reported to be major concerns in food including spices and dehydrated onion products reported by Savitha *et al*. Oludairo *et al*. also reported methicillin-resistant *Staphylococcus aureus* (MRSA) in cattle milk from dairy cattle in Ibarapa, Oyo and Oke-Ogun areas of Oyo State, Nigeria, multidrug-resistant (MDR) *Salmonella* species from locally fermented ready-to-take (RTT) milk (nono) from Gambari Market, Ilorin East Local Government Area of Kwara State, Nigeria and antimicrobial-resistant phenotype *Salmonella* species from ready-to-eat (RTE) roasted meat (suya) from Ilorin metropolis, Kwara State, Nigeria.

DISCUSSION

The highest number of respondents recorded at the Veterinary Teaching Hospital may be because the institution is a tertiary government-owned establishment since the government is the highest employer of labour in the part of the world. The high proportion of respondents with knowledge of food security may be due to their level of education and knowledge in the medical laboratory field. At the same time, respondents' age could play a role too since the younger respondents with perceived knowledge all had university education with a good proportion being veterinarians/medical practitioners. The high proportion of respondents that stated they were not stakeholders in the food industry could be because of the perception that laboratory staff are not direct mainstream food industry workers although they are involved in food testing and quality control.

Food may contain pathogens that may or may not be resistant to antimicrobial agents (**Murali *et al*., 2023**). An AMRM changes over time and does not respond any longer to antimicrobial agents; the state makes it harder to treat infections, leading to increased risk of spread of disease, severe illness, and death (**WHO, 2021**). Food may be contaminated with these microflorae from harvesting through preparation to consumption. The types of microorganisms and their natural levels in food play important roles in public health, food storage life, and spoilage (**Erkmen and Bosoglu, 2016**). There are published reports of food contamination by antimicrobial-resistant and non-resistant microorganisms (**Gholami-Shabani *et al*., 2023**). Research demonstrated that AMRM infection(s) more frequently occur in low- and middle-income countries (LMICs) compared to developed countries (**Samtiya *et al*., 2022**).The reports of the presence of AMRM in food by **Faleke *et al*., (2017); Savitha *et al*., (2022); Okanlawon *et al*., (2023) and Oludairo *et al*., (2020; 2022; 2023)** are an indication that contamination of food by AMRM could occur and that it could be throughout the food chain.

Food contaminated with antimicrobial-resistant or non-resistant microorganisms is unwholesome for consumption by man and animals. It could also result in food spoilage/wastage and consumer/customer dissatisfaction **(Alegbeleye *et al*., 2022).** This may have socio-economic implications of food shortages, food wastage, hunger, and increased greenhouse emissions from food deterioration **(Jeswani *et al*., 2021).** There could be an increase in the number of hospitalizations/risk of invasive infection and mortality (**Verraes *et al*., 2013**), in addition to resultant medical treatment failure, limited choice of antimicrobial agents for treatment, and higher risk of increased bacterial virulence through the integration of virulence and resistance plasmids **(Fluit, 2005).**

Antimicrobial-resistant microorganisms can develop and contaminate food through indiscriminate/improper use of antimicrobial agents during agricultural production, possible presence of resistant bacteria in probiotics, starter cultures, bacteriophages, and bio-conserving microorganisms that are added intentionally during food processing, and cross-contamination of antimicrobial-resistant bacteria from the environment, utensils/equipment, animals and handlers' hands **(Verraes *et al*., 2013).**

To avoid AMRM in food, there should be a limitation of microbial contamination of animal food products and the primary plant, which can be achieved by stakeholders in the food industry through adherence to Good Agricultural Practices **(GAP) (Verraes *et al*., 2013).** The compliance of stakeholders with good manufacturing practices (GMP) and good hygienic practices (GHP) is indispensable in achieving safe food production from farm to fork. Among other things, this may be achieved by the observation of physical parameters like temperature and time in heat treatments and thorough cooking before food consumption (**Cheng, 2022**). Cleanliness, hygiene, and preventive measures like the application of a Hazard Analysis

Critical Control Point (HACCP) can reduce the occurrence of AMRM in food. Special attention should be given to reducing the presence of AMRMs like *Salmonella, E. coli, and Campylobacter* in food through proper isolation, close monitoring of the process to prevent contamination, and implementation of active surveillance systems **(Molbak, 2005; Thapa *et al*., 2020).**

Isolated microorganisms should be tested for antimicrobial resistance; an AMR surveillance scheme should be implemented along the food chain **(Jans *et al*., 2018).** Testing of microorganisms like probiotics and starter cultures that are intentionally added to foodstuffs in the course of production to ensure they are AMRM-free is important in avoiding these organisms in food **(Verraes *et al*., 2013).** Antimicrobial Sensitivity Testing (AST) should be carried out by stakeholders in accordance with prescribed standards, especially when using the disc diffusion method which is the gold standard **(Hoyt and Levine, 1947; Khan *et al*., 2019).** These standards include the use of freshly cultured discrete colonies, equilibrating the turbidity of the culture solution to 0.5 McFarland, and evenly inoculating prepared microorganism solution on the plate using a sterile swab **(WHO, 1961).** Others include the use of standardized antimicrobial discs and ensuring proper placement on the AST plate, correct measurement of the zone of inhibition diameter after incubating for 24 hours, and interpretation of the measurements based on the recommendation the Clinical and Laboratory Standard Institute or other chosen international standards **(Reller *et al*., 2009; Khan *et al*., 2019).**

Bacteriophages or "bacteria eaters", are viruses that infect bacteria and destroy them. They can be used as food bio-preservatives to control food AMRMs, including other food preservation and processing techniques that can reduce the number of AMRM in foodstuffs **(Verraes *et al*., 2013).** Antimicrobial use should be controlled throughout food production in animal and crop agriculture to reduce its indiscriminate/improper use **(Samtiya *et al*., 2022).** This is more so in food of animal origin like poultry meat, eggs, pork, fish, and beef which are thought to be primary sources of AMRM **(Samtiya *et al*., 2022).** There should be increased public awareness through active communication, education and training **(OIE, 2019).**

Governments and industries in the food sector should provide funding for cutting-edge research in this area while laboratorians and researchers in the universities and other research institutes should engage more in the area of AMRM in food with the extension of their research findings to concerned stakeholders outside the university walls. Acquired knowledge by concerned stakeholders should be transformed into practice for the common benefit of the public **(WHO, 2015).** Acknowledging the inextricable link between human health, animal health and the environment, better collaboration among stakeholders is needed to reduce AMRM in food **(UNEP, 2023).** This should involve policymakers, regulatory bodies, food producers, animal health practitioners, pharmaceutical companies, academics, and research institutes **(Pokharel *et al*., 2020).** The World Health Organization (WHO), the Food and Agricultural Organization (FAO), and the World Organisation for Animal Health (OIE) proposed a one-health, multidisciplinary, multisectoral approach to avoiding AMRM in food **(WHO, 2015).**

CONCLUSION

Food is important in the provision of essential nutrition to man and animals. The indicators of a food-insecure country are when the populace lives in hunger and fear of hunger. Food security in Nigeria will be achieved through collaborative efforts of stakeholders, especially in the prevention of AMRM like *Staphylococcus* spp., *Proteus* spp., *Salmonella* spp., and *E. coli* in food. These organisms contaminate food, making it unwholesome for consumption and further reducing the amount of food available to the masses. Education and awareness of the public/food consumers will keep food producers and retailers on their toes to avoid improper use of antimicrobial agents during production and prevent contamination of food by ASRM. Regular food product testing and laboratorians' adherence to standards of AST to be able to obtain reliable results on which informed decisions can be made are other preventive measures that could be employed. More research works that give back to the community in the area of AMRM avoidance in food will produce results that can be extended to concerned stakeholders while the government provides resources and a conducive environment to be able to achieve better food security in the interest of the public.

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Conflict of interest

The authors declare that there is no conflict of interest

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